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THE VISCOSITY OF THE BLOOD ON INHALATION OF ALCOHOL

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It has been shown by me[1] that grain alcohol, when introduced into the alimentary tract or into the vascular channels directly, produces a considerable increase in the viscous resistance of the circulating blood. In amplification of these experiments the present tests have been undertaken to determine whether this result can also be attained by inhaling the vapors of grain alcohol for a considerable length of time, and secondly, whether wood alcohol possesses an action similar to that of grain alcohol.

The experiments which I am about to submit are divided into two parts and present a number of determinations of the normal viscosity of the blood in relation with a number of tests made during the period of inhalation of the alcohol. Having ascertained the normal coefficient in the manner repeatedly described by me, the dogs used in these experiments were made to breathe through a large bottle containing a relatively small quantity of either grain alcohol or wood alcohol. The preliminary tests were made during light ether narcosis, after which the tracheal cannula of the animal was connected with the bottle by means of a short tube fastened in such a way that its end came to lie close to the surface of the alcohol. The agitation

[1] Journ. of Physiol., xxxii, 8, 1904.

of the surface-layers of the latter by the respiratory current was deemed sufficient to charge the air with alcohol. An adequate depth of narcosis having been attained within a few minutes after the beginning of the inhalation, the ether was discontinued altogether; in fact, it frequently became necessary to substitute fresh air, because the initial increase in the frequency of the heart and the rise in the systemic blood pressure repeatedly gave way to vascular depressions. In spite of this precaution two of the animals died before the sixth determination could be made, or in less than an hour after the beginning of the inhala-

Viscosity of blood on inhalation of alcohol

		DETERM. NO.	DURATION OF INHALATION	SPEC. GRAVITY OF BLOOD	RECT. TEMP. °C.	QUANTITY	TIME	PRESSURE	VISCOSITY COEFFICIENT	AVERAGE VALUES	GREATEST DIFFERENCE
			min.			*mgm.*	*sec.*	*mm.Hg*			
Exp. 1 (wood alc.)	Normal	1		1.0536	36.9	494.7	32.64	166.6	886.20	860.0	213.6
		2				378.6	35.50	124.6	833.80		
	After inhal.	3	50	1.0609		417.8	33.84	177.1	674.39	646.4	
		4	65			347.0	32.17	168.7	618.52		
Exp. 2 (grain alc.)	Normal	1		1.0517	37.0	493.3	40.73	133.3	888.68	866.0	207.6
		2				529.8	43.85	138.3	852.71		
		3				562.9	45.29	141.6	856.76		
	After inhal.	4	50	1.0540		487.0	42.70	159.5	696.32	658.4	
		5	65			310.4	52.09	93.5	620.62		

tion. One of them had been given grain alcohol and the other wood alcohol.

The results of these tests are clearly portrayed by the two experiments given in the accompanying table. The first animal received wood alcohol and the second grain alcohol. In each case the normal viscosity of the blood is contrasted with the viscosity prevailing fifty and sixty-five minutes after the beginning of the inhalation of the alcohol. The normal coefficients which show the numerical values 860.0 and 866.0, indicate that the blood of these animals possesses a viscous resistance which is 5.4 times greater than that of distilled water at 37°C. Fifty

minutes after the beginning of the inhalation of the alcohol the coefficients exhibit a much smaller value which fact suggests that the viscosity has been considerably increased. Thus, if the viscosity of distilled water is taken as the standard, the coefficients 674.3 and 696.3 prove that the inner friction of the blood has become almost 7 times greater. An additional rise resulted within the following fifteen minutes, because the coefficients 618.5 and 620.6 obtained sixty-five minutes after the beginning of the inhalation, indicate a viscous resistance of the blood 7.5 times greater than that of the standard fluid.

The normal ratio of 5.4: 1, when contrasted with the ratio of 7.5: 1 obtained within one hour after the beginning of the inhalation, clearly shows that alcohol administered in the respiratory air markedly increases the viscous resistance of the circulating blood. The examples submitted in the accompanying table prove moreover that grain alcohol exerts as powerful an influence as wood alcohol and hence, it cannot be said that the latter is more closely associated with the group of symptoms ordinarily encountered in the course of poisoning by this agent. The general appearance of the blood, as well as the decided increase in its specific gravity, may be taken to indicate that the change in its viscosity is due to an accumulation of excretory material rather than to a direct destructive action of the alcohol upon the cellular constituents of the blood. It may also be concluded that these changes result very soon after the beginning of the inhalation.

FACTORS OF INFLUENCE IN THE ORIGIN AND CIRCULATION OF THE CEREBROSPINAL FLUID

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Of the many problems of surgical and medical research, few are more pregnant with possibilities of real value than those dealing with the physiology of the cerebrospinal fluid in health and disease. The accumulation of cerebrospinal fluid in abnormal quantities is a factor which must be reckoned with in many intracranial diseases. It is accountable not only for many subjective and objective disturbances, but it is often the determining factor in the fatal cases. How essential it is in diagnosis, prognosis, and treatment will be realized by the mere mention of such morbid conditions as hydrocephalus, brain tumors, and meningitis. In the treatment of these, so far as it concerns the damaging effect of excessive secretion or inadequate absorption of cerebrospinal fluid, we are helpless and woefully unsuccessful. It is of the utmost importance, therefore, that investigation of the cerebrospinal fluid should be continued until the fundamental principles of secretion and absorption are thoroughly understood in the hope of finding some effective means of controlling over-secretion or diminished absorption.

The following experiments were conducted for the purpose of clearing up some disputed points and of making further contributions to our knowledge of the cerebrospinal fluid:

1. The artificial production of hydrocephalus.
2. Normal cerebrospinal pressure and variations due to circulatory changes.

3. Normal rate of secretion, and influence of circulatory disturbances.

4. Rate and mode of absorption under normal and pathological conditions.

It is now generally accepted that the cerebrospinal fluid is secreted by the choroid plexus of the various ventricles. Faivre[1] in 1853 first suggested the secretory property of the cubical cells covering the choroid plexus, while Luschka[2] advanced the same theory two years later. Since that time numerous workers have given added strength to the theory. The recent work of Mott[3] strongly supports by histological evidence the secretory nature of the choroid plexus. He believes the cerebrospinal fluid has a double function, first as a nutrient fluid for brain tissue, and secondly as an excretory product, receiving the waste products of brain metabolism, being somewhat analogous in this respect to the lymph.

Early workers attempted to show by chemical analysis that the cerebrospinal fluid was a secretion rather than a transudate from direct filtration, as Schmidt[4] who called attention to the larger proportion of potassium salts in lymph than in cerebrospinal fluid.[5] On the other hand, the careful chemical analyses by Halliburton, Mott and others[6] show the inorganic constituents of cerebrospinal fluid to be almost identical, both qualitatively and quantitatively, with the salts found in the blood plasma. It is thus seen that by chemical analysis alone, we cannot prove cerebrospinal fluid to be a true secretion, but the histological evidence in connection with the experimental data we wish to present proves quite conclusively that the cerebrospinal fluid is a true secretion of the choroid plexus.

[1] Faivre: Ét. s. l. conarium et les plexus chor. chez l'homme et les animaux. Ann. d. sc. natur, 1853.

[2] Luschka: Die Adergeflechte des menschlichen Gehirns. Berlin, 1855.

[3] Lancet: July 2–9, 1910.

[4] J. sc. méd. de Lille, xi, 603.

[5] Folia: Neuro-Biologica Bd. I, 1907–1908.

[6] Findlay: Jour. of Mental Science, xliv, 1898. Pettit and Girard. Comptes Rendus de la Soc. de Biologie, July 27, 1901, June 14, 1902.
Cavazzani: Archives Italiennes di Biologie, vol. xxxvii, p. 30. La Riforma Medica, viii, 1892. Capilleti: Academia Medico-chirurgica di Ferrara, 1900.

The fluid is almost entirely secreted within the two lateral, the third and the fourth ventricles, and reaches the general subarachnoid space through three openings. These are situated at the posterior end, and on either side of the lateral recesses of the fourth ventricle, named respectively, the foramen of Magendie and the foramina of Luschka, the lateral and third ventricles being connected with the fourth by a single narrow passage, the aqueduct of Sylvius.

Observation I. Artificial hydrocephalus. Many of the problems relating to the secretion and the absorption of cerebrospinal fluid have remained obscure because of the widespread distribution of the cerebrospinal fluid throughout the subarachnoid and subdural spaces of the brain and cord. Of great service in the solution of these problems has been the production of an artificial hydrocephalus. This condition we have succeeded in producing experimentally by mechanically plugging the lower end of the aqueduct of Sylvius or by effecting its closure through the medium of inflammatory products which are the result of chemical irritation. In our experimental work, we used each method separately and a combination of the two.

The technic of the first method was as follows: a median incision from the occipital protuberance to the third or fourth cervical vertebra through skin and muscles exposed the occipito-atlantal ligament. When this and the underlying dura were incised, the cerebrospinal fluid spurted out with considerable force. (In young dogs the foramen of Magendie and the vermes of the cerebellum can be seen through this opening, while in older animals it is necessary in order to expose these structures to remove the bony ridge which projects downward from the foramen magnum). The vermes was then slightly elevated and a plug of gauze or cotton passed through the foramen of Magendie into the fourth ventricle, where, in contact with the lower end of the aqueduct of Sylvius, it blocked the outlet. At first there is some leakage through the gauze or cotton, but the inflammatory exudate soon plugged their meshes. The animals showed no signs of increased intracranial pressure for four or five days, but then they became more quiet, preferred to lie down or sleep, and seemed less conscious

of their surroundings. In two or three weeks it became evident that their nutrition was disturbed by the loss of weight and strength, later the limbs became spastic and soon the animals became comatose. In this state they would respond slightly to touch, but appeared to have no higher cerebration, and after two or three days of absolute unconsciousness, the animals died. Autopsy always revealed marked dilatation of the ventricles.

In the second method, the foramen of Magendie was exposed in like manner, and 1 to 2 cc. of a suspension of aleuronat injected into the fourth ventricle, a considerable amount of the aleuronat passing through the aqueduct of Sylvius into the third ventricle. The results were the same by this method, though on the whole not so satisfactory, as in some instances the aleuronat did not produce sufficient irritation to effectively block the outlets.

In a few dogs the aleuronat was introduced into the lateral ventricle through a small trephine opening, and the results were essentially the same, although occasionally only one lateral ventricle was dilated.

The most striking results were obtained by combining the two methods. The aleuronat suspension was injected through the fourth ventricle into the third and a gauze plug placed against the lower end of the aqueduct of Sylvius. This rapidly produced an artificial hydrocephalus. If 1 cc. of a thick aleuronat suspension was injected, and the gauze plug introduced, the resulting hydrocephalus was so acute that most of the animals died in from twenty-four to thirty-six hours, though some survived two or three days, but they all showed an early loss of the higher cerebral functions as evidenced by stupor, loss of appetite, etc. At the same time muscular weakness was apparent, and a few hours before death the legs were often spastic. While some animals passed into a comatose condition as early as twelve hours after operation and remained so until death twelve or more hours later, most of the dogs did not become absolutely comatose until two or three hours before death, and none recovered. The autopsy revealed a moderate hydrocephalus, but no signs of meningitis were observed in any instance.

By the production of an artificial hydrocephalus, it becomes possible, as we will see later (see page 281), to differentiate between

various types of hydrocephalus, and to demonstrate not only that the cerebrospinal fluid is generated only within the ventricles, but that absorption takes place only after the fluid leaves the ventricles.

Observation II. Pressure of cerebrospinal fluid. The normal pressure of the cerebrospinal fluid varies as the pressure in the venous sinuses and is practically identical with it. The pressure oscillates under certain normal and certain abnormal conditions. Then there are oscillations in the cerebrospinal pressure due to expansion of the cerebral arteries, but these are very small since the force of the arterial pulse is expended largely in forcing the blood from the capillaries and veins into the venous sinuses. The respiratory oscillations are much more marked and are due to the variations of pressure in the right side of the heart. As there are no efficient valves intervening between the cerebral sinuses, and the vena cava, the blood is dammed back into the sinuses during expiration, thus slightly raising the cerebrospinal pressure, while during expiration the reverse is true, as the suction action of the thorax favors the venous circulation from the sinuses to the vena cava. In the dog this respiratory oscillation is normally about 2 mm. of water; during very forced breathing it may rise and fall double this distance, and if the breathing is shallow the pressure is lower. We found, however, that the inspiratory rise in blood pressure caused more marked intracranial tension (with resulting increase in cerebrospinal fluid pressure) than the expiratory rise in the venous pressure. Thus character and extent of the oscillations in pressure of the cerebrospinal fluid depend entirely upon the depth of the respiratory movements and to a limited extent upon the magnitude of the cardiac impulse.

Sufficient intracranial pressure can easily be made to arrest these respiratory and cardiac oscillations. This we demonstrated in two ways. (1) Under ether anesthesia a large tube covered at its inner end by a thin rubber bag was secured in a trephine opening, and when the bag was distended with air or water, the oscillations stopped immediately. (2) A needle was inserted into the subarachnoid space and attached to a burette. When a column of water was introduced into the burette, under a pressure only slightly in excess of the venous pressure, the oscillations were arrested.

While normally the cerebrospinal fluid is secreted under a pressure equal to the cerebral venous pressure, when there is a free escape of fluid, as in cases of basal fracture, the pressure falls, even to zero. Under such circumstances the fluid must be formed under a pressure lower than in the veins of the choroid plexus, so that the question arises as to whether the fluid under these conditions may not pass through the choroid plexus as a transudate. This may explain the enormous amount of fluid which may escape from cerebrospinal fistulae, and the diminution in the total solid, which has been proven by chemical analysis, adds weight to this assumption.

The effect of increase in the fluid pressure in different parts of the cerebrospinal system is interesting and may explain some of the cases of hydrocephalus due to brain tumors. We found that if a column of fluid is introduced through a needle inserted into the cisterna magna, at a pressure considerably in excess of the normal venous pressure, the mid-brain is forced upward blocking the isthmus of the tentorium cerebelli. This prevents any fluid passing from the posterior fossa forwards over the cerebral cortex. This phenomenon was further demonstrated by exploring through a trephine opening the parietal or frontal region; in neither region could any of the fluid introduced into the posterior fossa be seen escaping over the cerebral cortex.

In this experimental observation we find a possible explanation of some of the phenomena we have observed in cases of brain tumor. It is not rare to find a tumor at the base of the brain, which does not press upon the aqueduct of Sylvius nor obstruct the foramen of Magendie, but which, nevertheless, is associated with a hydrocephalus, and it seems reasonable to assume that the tumor may have elevated the mid-brain sufficiently to block the passage through the tentorium cerebelli. Since, as we will show later, the cerebrospinal fluid is absorbed from the entire subarachnoid space, the exclusion of so large an absorptive area must result in one of two conditions: either the fluid will have to be absorbed much more rapidly from the remaining area or a hydrocephalus will result.

As the pressure of the cerebrospinal fluid is under normal conditions identical with venous pressure, conditions affecting venous pressure, such as disturbances of arterial pressure or obstruction to the venous ouflow, should disturb the pressure of the cerebrospinal fluid. A number of observations were made to confirm this hypothesis, and to this end many records were made on a water manometer attached to a cannula introduced into the cisterna magna. Thus when both carotid arteries were clamped, the pressure fell from 90 mm. to zero in sixteen seconds. But with the clamps still in place, the pressure rose in four and one-half minutes to 30 mm., and upon removing the clamps the pressure rose in five seconds to 90 mm. or about normal where it remained. Clamping the right or left carotid separately caused a drop of 30 mm. in five seconds.

On clamping one internal jugular vein, there was no change in the pressure of the cerebrospinal fluid, but on clamping both internal jugular veins, the pressure rose 20 mm. in four seconds to return to normal in five minutes after the clamps were removed. Ligation of both internal and the right external jugular vein caused a rise of 10 mm. in three seconds, and when the left external jugular vein was closed, the pressure rose 40 mm. in ten seconds. When with both external and internal jugulars closed, both common carotid arteries were clamped, the pressure fell almost to zero in fifteen seconds, rising to 20 mm. in one minute, to 30 mm. in another minute and a half, and to 40 mm. in the next ten minutes.

In other words, the pressure of the cerbrospinal fluid drops to zero after occlusion of the carotid circulation only to recover itself in a very few minutes, while occlusion of the venous circulation is followed by a rise in pressure more or less constant. The rapid recovery of pressure after disturbance of the arterial circulation is a more striking feature of the experiment, and is explained, as we will see later, by the trifling influence upon the rate of secretion which is exerted by arterial occlusion. The more or less constant rise in pressure when the venous circulation is interrupted confirms the hypothesis that the absorption of cerebrospinal fluid takes takes place chiefly through the venous channel.

Observation III. Rate of secretion. In order to determine the factors which may influence the secretion of cerebrospinal fluid, it is necessary to establish first within a reasonable range the normal rate of secretion. These observations were made on the dog, always under anesthesia, and the animals were killed at the end of the experiment. The most suitable anesthesia was found to be preliminary chloroform or ether narcosis followed by the maximum dose of urethane, one-eighth to one-fourth grain of morphine and five grains of urethane being sufficient in most instances for a dog weighing 5 or 6 kilos.

After a trial of many methods, the technic finally adopted for determining the rate of normal secretion was as follows: The occipito-atlantal ligament was exposed and through a small incision a blunt cannula was inserted and sutured in place, and to this a rubber tube and graduated pipet were attached. To recover all the fluid secreted, it was necessary to place the animal in such a position that the fluid would flow from the cannula as fast as it escaped from the foramen of Magendie. Otherwise some would be absorbed from the subarachnoid space. This was accomplished by placing the subject on an inclined plane, head down, so that the outlet of the cannula through the occipito-atlantal ligament was at the most dependent point. A large number of readings under normal conditions were taken, using dogs of different weights of which the following are types:

I. Dog, weighing 10 kilos. Five minutes after complete drainage of cisterna magna, the fluid was flowing into pipette at the following rate:

TABLE I

0.2 cc. in 40 seconds	0.2 cc. in 30 seconds
0.1 cc. in 15 seconds	0.1 cc. in 30 seconds
0.1 cc. in 15 seconds	0.04 cc. in 20 seconds
0.1 cc. in 15 seconds	0.1 cc. in 20 seconds
0.1 cc. in 15 seconds	0.1 cc. in 25 seconds
0.1 cc. in 30 seconds	0.1 cc. in 25 seconds
0.1 cc. in 20 seconds	0.1 cc. in 40 seconds
0.1 cc. in 30 seconds	0.02 cc. in 60 seconds

Summary: 1.66 cc. in 7¼ minutes.
Average, about 0.231 cc. per minute.

II. Dog, weighing 8 kilos.

TABLE II

0.10 cc. in one minute	0.11 cc. in one minute
0.11 cc. in one minute	0.06 cc. in five minutes
0.05 cc. in one minute	0.05 cc. in one minute
2.0 cc. in four minutes	0.1 cc. in one minute
0.19 cc. in one minute	Stopped flowing and dog killed with chloroform.
0.8 cc. in two minutes	

Summary 3.57 cc. in 18 minutes.
Average, 0.192 cc. per minute.

When the animal was placed with the head elevated, the reverse of the position in the previous experiment, the rate of flow from a cannula introduced in the dural sac of the lumbar region was only one-half to one-third as fast as that from the cisterna magna. We attribute this difference to the fact that in the latter experiment a greater opportunity for absorption was offered than when the fluid was drawn directly from the cisterna magna.

It has been suggested that the secretion of cerebrospinal fluid may be controlled by ligation of the common carotid arteries, and in fact this operation has been recommended in the treatment of hydrocephalus. Inasmuch as the number of clinical observations are very limited, it seemed desirable to investigate the effect of disturbance of the cerebral circulation upon the rate of secretion in order, if the results were confirmatory, to place the operation upon a sounder basis. The following observations were made, therefore, upon the rate of secretion after clamping first one common carotid artery, then both:

TABLE III

0.03 cc. in one minute	0.03 cc. in one minute
0.04 cc. in one minute	0.03 cc. in one minute
0.03 cc. in one minute	0.03 cc. in one minute
0.02 cc. in one minute	0.03 cc. in one minute
0.08 cc. in two minutes	0.02 cc. in one minute
0.03 cc. in one minute	0.02 cc. in one minute
0.04 cc. in one minute	0.03 cc. in one minute
0.03 cc. in one minute	Unclamped left carotid
0.04 cc. in one minute	0.02 cc. in one minute
Clamped left carotid	0.02 cc. in one minute
0.03 cc. in one minute	0.03 cc. in one minute
0.03 cc. in one minute	Both carotids unclamped
0.02 cc. in one minute	0.03 cc. in one minute
0.02 cc. in one minute	0.02 cc. in one minute
0.03 cc. in one minute	0.03 cc. in one minute
0.03 cc. in one minute	0.01 cc. in one minute
0.03 cc. in one minute	0.02 cc. in one minute
0.02 cc. in one minute	0.01 cc. in one minute
0.02 cc. in one minute	0.02 cc. in one minute
0.02 cc. in one minute	0.01 cc. in one minute
0.03 cc. in one minute	0.02 cc. in one minute
0.03 cc. in one minute	0.02 cc. in one minute
0.03 cc. in one minute	0.01 cc. in one minute
0.03 cc. in one minute	
Both carotids clamped	
0.04 cc. in one minute	
0.02 cc. in one minute	

I had an opportunity of making similar observations on two cases of brain tumor with hydrocephalus, and obtained essentially the same results. In one case an exploratory craniotomy was performed, but the tumor proved to be inoperable. The enormously distended ventricles elevated the osteoplastic flap sufficiently to make it possible to introduce the cannula through the scalp directly into the ventricles. The latter were tapped on several occasions; the cerebrospinal pressure was recorded before and after the withdrawal of measured amounts of fluid, and the time taken for the ventricles to refill was noted. Similar observations on the cerebrospinal pressure were made both before and after ligation, first of one and then of both common carotid arteries. No definite change either in the cerebrospinal pressure

nor in the time taken for the ventricles to refill occurred. It would seem, therefore, from the experimental data and from clinical observations that ligation of the common carotid arteries should not be expected to influence favorably the hydrocephalus, either of congenital origin or the accompaniment of brain tumors, and that this operation should not be endorsed, at least until contradictory evidence is forthcoming from other sources. It will be recalled that our experimental observations showed a sudden fall in cerebrospinal pressure when both carotids were clamped, but this was quite transitory, as the pressure soon returned to normal.

It is not to be expected that disturbance of the venous circulation would in any way affect the secretory output of the choroid plexus, as the venous channels are concerned with absorption rather than with secretion, and when a free outlet was afforded the cerebrospinal fluid through a cannula introduced into the cisterna magna, the effect on absorption could not of course be estimated. However, in estimating the rate of secretion from closure of the carotid arteries in the observation just recorded, an opportunity was taken to record the outflow of cerebrospinal fluid after closure of the internal jugular and the results, as anticipated, were negative.

TABLE IV

Left jugular vein clamped	Both jugulars unclamped
0.02 cc. in one minute	0.03 cc. in one minute
0.10 cc. in one minute	Left carotid unclamped
0.06 cc. in one minute	0.03 cc. in one minute
0.04 cc. in one minute	0.02 cc. in one minute
0.06 cc. in two minutes	0.02 cc. in one minute
Both jugular veins clamped	Both internal jugulars clamped
0.03 cc. in one minute	0.02 cc. in one minute
0.02 cc. in one minute	0.02 cc. in one minute
0.03 cc. in one minute	0.03 cc. in one minute
0.02 cc. in one minute	0.07 cc. in five minutes
Left carotid clamped	0.02 cc. in one minute
Jugulars remain clamped	0.02 cc. in one minute
0.02 cc. in one minute	0.02 cc. in one minute
0.03 cc. in one minute	0.03 cc. in two minutes
0.02 cc. in one minute	

An exception was noted when the position of the animal was reversed so that the point of entrance of the cannula was higher than the head. Under these circumstances, upon clamping both internal jugular veins *the rate of flow practically doubled.* This increase in flow may be explained in one of two ways: the obstruction to the venous outflow dilates the sinuses and forces out some of the cerebrospinal fluid stored in the ventricles—a merely transitory phenomenon; or the rate of absorption is lowered by the closure of the venous channels.

Pressure upon the sinuses and obstruction to the venous circulation is no doubt a factor in the accumulation of cerebrospinal fluid so frequently associated with brain tumors, especially of the posterior fossa. As the major portion of the venous circulation of the brain, including the cerebral veins and dural sinuses, leaves the cranial cavity from a point in the posterior fossa, the jugular foramen, it is readily understood why posterior fossa tumors are so frequently and at a comparatively early stage associated with increased intracranial tension which comes from the accumulation of cerebrospinal fluid.

Observation IV. Rate and mode of absorption under normal and pathological conditions. Of the two theories which have been advanced to explain the mode of absorption of cerebrospinal fluid, the predominance of evidence favors the venous rather than the lymphatic channels as providing an outlet for the bulk of the fluid. There remain some who claim that there has been no positive proof of this, but our own observations support the majority rather than the minority point of view.

Lymphatic absorption. To determine the extent of absorption by the ymph channels, we injected into the ventricles or subarachnoid space various dyes, including methylene blue, isomine blue, trypan red and trypan blue, and noted the time of their appearance and distribution in the blood and lymph glands. Under ether anesthesia the occipito-atlantal ligament was exposed, a fine hypodermic needle inserted into the subarachnoid space and 1 to 2 cc. of a 1 per cent. saline solution of the dye injected slowly. To prevent leakage, which occurred sometimes on the withdrawal of the needle, 2 or 3 cc. of the cerebrospinal

fluid were withdrawn before the dye was injected. In a second series of animals the dye was injected into the spinal subarachnoid space through a lumbar puncture, and a third series differed only in that the dye was injected into one of the lateral ventricles through a small trephine opening.

From six to twelve hours after the injection, the animals were killed with chloroform and a careful inspection made. The course taken by the stain was practically uniform in every instance. It passed first through the nerve sheaths, principally of the optic and olfactory nerves, and then to the tissues outside the skull especially in the posterior part of the orbit and the posterior portion of the mucous membrane on the turbinates and septum, which was always deeply stained. The dye was traced then to the lymphatic trunks as far as the nodes in the posterior naso-pharynx. If the animal was killed at a later hour the parotid group of lymph nodes was involved, and later still the dye was found to have passed through the cervical chain of lymph nodes to the thoracic duct, and finally to the great vessels in the thorax. The glands in no other part of the body showed any staining.

The significant feature of this study of lymphatic absorption was not the fact that the lymphatic system was found to play a part in absorption, nor the fact that the course of the stained fluid was uniform, but the time which elapsed between the injection and staining of the cervical lymph nodes. Even with trypan blue, which traveled the fastest, almost twelve hours were required in the passage of the dye-impregnated cerebrospinal fluid from the subarachnoid space only as far as the cervical chain. When we compare this with observations about to be recorded, there can be, or should be, no doubt in the minds of the most critical that the lymphatic system is almost a neglible factor as a source of absorption.

Venous absorption. The best method for studying the absorption of the cerebrospinal fluid is undoubtedly the injection of phenolsulphonephthalein.[7] When phthalein is injected into the

[7] Our observations upon the absorption of cerebrospinal fluid based upon the phthalein test were completed before we heard of the splendid work of Dandy and Blackfan, who had used the same substance by exactly the same method and with precisely the same results.

ventricles of a normal dog, it appears in the blood of the torcular herophili in two to three minutes. It appears in the urine caught from a divided ureter in five and one-half minutes and could always be demonstrated in bladder urine in from six to ten minutes. If injected into one of the lateral ventricles, it appeared in the subarachnoid space almost immediately and in the blood and urine in practically the same time as given above.

When we produced an experimental hydrocephalus by blocking the fourth ventricle, the absorption from the ventricles was negative. Even after two hours, only the slightest trace could be found in the urine and this was not constant, whereas under normal conditions about 50 to 60 per cent. is excreted into the bladder within two hours. By this method, it would be possible to differentiate the types of hydrocephalus, those with obstruction and those without obstruction of the ventricles, and as a matter of fact, Dandy and Blackfan[8] have already applied it clinically with satisfactory results.

If we can assume that the cerebrospinal fluid is absorbed proportionately as rapidly as the amount of phthalein injected,and there is no reason for believing otherwise, we are lead to the conclusion that at least 50 or 60 per cent. of the cerebrospinal fluid is absorbed every two hours.

Résumé. The problem of absorption and secretion has been elucidated somewhat by the production of artificial hydrocephalus, so that it can be demonstrated without question that the cerebrospinal fluid is almost, if not entirely, secreted within the ventricles, and that absorption does not take place until the fluid has escaped. The lateral ventricle, therefore, is, as the bladder, merely a receptacle for the storage of fluid. Comparing the results of phthalein injection with and without obstruction of the lateral ventricles, it has been possible to elaborate a method for the differentiation of different types of hydrocephalus from the standpoint of etiology.

The cerebrospinal pressure has been found to vary with the pressure of the venous sinuses and to be practically identical with

[8] Dandy and Blackfan: Jour. Am. Med. Ass., Dec. 21, 1913.

it. Under normal conditions, there are certain oscillations, which are due to the effect of inspiration and expiration upon venous pressure, but they may be arrested by slight increase of intracranial tension. Closure of the carotid circulation causes an immediate but only transitory fall of cerebrospinal pressure, but obstruction of the venous circulation is followed by an increase in pressure more or less constant.

The normal rate of secretion was found to vary widely under normal conditions and was practically uninfluenced by such profound circulatory disturbances as complete closure of both common carotid arteries. The propriety of ligation of the common carotid arteries, recommended and practiced in cases of hydrocephalus, has not been established by exact laboratory methods.

The absorption of cerebrospinal fluid is largely by the venous channels. Lymphatic absorption is roundabout, slow, and relatively diminutive. By the phthalein test, it was proven that from 50 per cent. to 60 per cent. is absorbed within two hours. Within two minutes of an injection of phthalein into the lateral ventricles, the drug may be recovered from the blood in the torcular herophili. Absorption was found to be chiefly via the venous channels so that obstruction to the venous outflow is immediately followed by a rise in venous pressure, by the accumulation of cerebrospinal fluid, and a resulting increase in intracranial tension.

EXTIRPATION OF THE TESTES AND VASO MOTOR IRRITABILITY

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Morphological changes as a result of castration have received much attention.[1] On the other hand, relatively little information has been secured concerning the effects of such operations upon the general functions of the body.[2] It appears certain that the influence of the genital glands is not confined to organs functionally related to them. The effects of castration upon blood pressure and irritability of the sympathetic nervous system seems to have been entirely neglected. The present work deals with these conditions. It is one of a series of studies now being made upon the ductless glands in relation to the sympathetic system.[3]

Experiments were made upon dogs of various sizes and breeds. The average weight was ordinarily between 10 and 12 kilos. Fully developed, aggressive animals were chosen in order that any depressive changes might be followed more easily during the period of observation. To avoid complications, morphine or other sedatives were not employed.

Blood pressure was taken from the femoral arteries. By careful manipulation it was possible to obtain two readings, taken at different times, from the same vessels and retain sufficient collateral circulation to supply the limb. An arterial reservoir cannula simplified the method of obtaining pressure readings.

[1] Biedl, Arthur: The Internal Secretory Organs, William Wood and Company, New York, 1913.

[2] Marshall, F. H. A.: The Physiology of Reproduction, Longmans, Green and Company, London and New York, 1910.

[3] Hoskins and Wheelon: This Journal, 1914, xxxiv, pp. 81, 172, 268.

After insertion into the artery, the cannula was filled with 10 per cent sodium citrate solution and connected with a mercury manometer and float. A vein was connected by a cannula and rubber tubing to a reservoir of physiological saline solution. To eliminate variability that might be introduced by changes in size and bore, the same cannulas were used for any given animal.

Having obtained the normal blood pressure at each operation the vaso motor irritability was determined by injections of adrenalin and nicotin. Definite amounts of these drugs were introduced by means of a hypodermic needle into the tubing connected with the vein. Upon opening the clamp above the cannula the drug was rapidly flushed in under a pressure of 2 feet of water. As standard doses, 0.5 cc. and 1 cc. of adrenalin in a dilution of 1:50,000, and nicotin, 1:2,000 parts were used throughout the work. At the end of each experiment the vessels were ligated and the incisions closed.

Two series of observations were made for the present report. In series A of six animals, two separate readings were made before castration. In series B of five animals, but one determination was made since reported experiments demonstrated the blood pressure and the reactions to adrenalin and nicotin to remain fairly constant for any normal dog.[4]

Castration was performed through a longitudinal incision lateral to the median line at the base of the scrotum. The skin was thereby laid open above the proximal portions of the testis and over the spermatic cord. The cord was brought into the opening and ligated. The testis freed from the scrotum was severed from the cord distal to the ligature. The opposite organ was delivered through an incision in the septum. The cord stumps were then replaced and the incisions closed with interrupted sutures. This method gave desirable results.

Upon completion of the experiments the graphs were measured and the results tabulated. The average blood pressure for any experiment was taken as normal. The pressor effect of each drug was measured from the highest point at the time of injec-

[4] Hoskins and Wheelon: 1914. Ibid. p. 81.

tion to the highest point in the pressor curve. The following conclusions were based upon thirty-three determinations from eleven anaesthetized dogs. In table I, are shown the comparative determinations obtained from the entire set of experiments.

TABLE I

Showing average blood pressure and average pressor effects of constant doses of adrenalin and nicotin before and after castration. Based upon thirty-three determinations from eleven anaesthetized dogs. All readings are expressed in millimeters of mercury

	SERIES	BEFORE CASTRATION	AFTER CASTRATION	VARIATION
		mm.	*mm.*	*per cent*
Blood pressure	A	118.4	110.6	
	B	115.6	114.4	
Adrenalin reactions	A	33.0	33.2	
	B	31.4	31.6	
Nicotin reactions	A	25.4	16.6	37.5 loss
	B	44.0	25.2	42.8 loss

Adrenalin 1 cc., 1:50,000 solution, nicotin 1 cc., 1–2000 solution.

Normal blood pressure is but slightly lowered as a result of gonadectomy. In series A the mean average of twelve determinations upon six dogs before castration was 118 mm. After the operation the pressure was 110 mm. showing a loss of 6.5 per cent. In series B the loss was negligible. In cut B of figure 2 is shown the lowered blood pressure, as well as the reaction to a standard dose of adrenalin after castration.

The irritability of the myoneural junction of the arterial walls was determined by injection of adrenalin. Irritability to adrenalin, within the limits of experimental error, was constant before and after castration.

Figure 1 represents a composite curve of series A, plotted from measured readings obtained by injections of 1 cc. of adrenalin. Readings before castration are shown by the continuous line. The mean maximum increase of pressure is 32 mm. The pressure then falls 26 mm. below normal from which it gradually returns to the initial tension. The broken line represents the results obtained after castration. The curve shows a mean

maximum pressure rise of 33 mm. and a depression of 13 mm. The 50 per cent loss in the depression is the only difference between the two curves. The significance of this is not known. Readings from series B gave similar results (see table I). Characteristic graphs are shown in figure 2. Cut A represents the pressure reactions of Dog No. 66 to 1 cc. of adrenalin, 1:50,000, on March 13, 1914. The animal was then castrated. On April

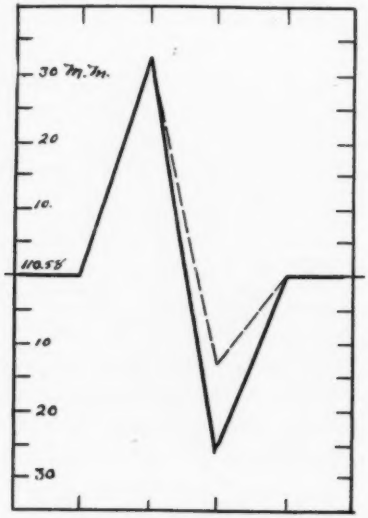

Fig. 1. Composite curves of series A showing blood-pressure responses to adrenalin. The continuous line represents reactions before and the broken line after castration.

7, 1914, the curve shown in cut B was obtained. The similarity of the two curves is marked. Castration did not appreciably alter pressor responses of the vascular system to adrenalin.

Nicotin was used as a vaso motor stimulant because of its selective action on the sympathetic nervous system. The responses of the vascular system to injections of standard doses of this drug show that castration lowers sympathetic irritability.

Figure 3 shows a composite curve of alterations in the blood pressure resulting from constant doses of nicotin. The contin-

uous line represents the mean values before castration. The curve shows a mean maximum pressor effect of 25.5 mm. The broken line shows results after the removal of the testes. The pressor effect reaches a mean maximum of 16.6 mm. from which it gradually returns to normal. This sustained pressor effect is characteristic. The difference in height of the two curves is 8.8 mm. or a difference of 37.5 per cent and represents the extent of the lessened irritability of the sympathetic nervous system. The first set of determinations before castration, in series A

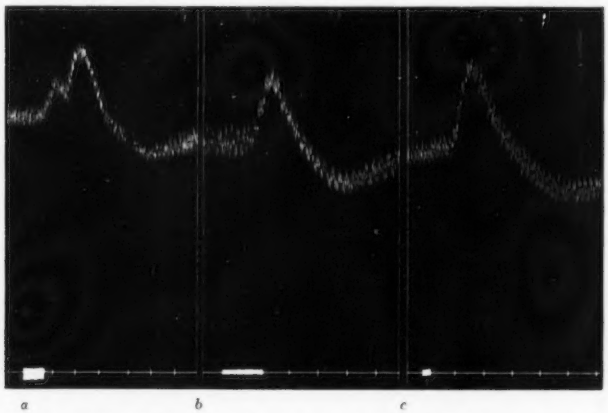

Fig. 2. Reactions of Dog No. 66 to 1 cc. of 1:50,000 adrenalin. *a*, March 13, 1914, before castration; *b*, April 7, 1914, after the operation, and *c*, April 10, 1914, after reception of testicular grafts. The graft was placed after obtaining reading *b*. Time, five seconds.

gave an average pressor effect of 25.5 mm. The second set averaged 23.3 mm. Therefore, inasmuch as the two sets of pressor readings before castration were within 1 mm. of each other it is evident that the operations for determining blood pressure had not altered vascular control. In figure 4 are shown characteristic curves demonstrating the decreased response to nicotin as a result of gonadectomy.

Testicular grafts were found to restore irritability in castrates practically to normal. Portions of testes were transplanted to

lacerated surfaces beneath the superficial abdominal muscles of dogs previously castrated. Readings were made from three to ten days after the operation. Tracing C of figure 4 shows the effect of a graft in the heightened response of the blood pressure to nicotin. Little variation occurred in the blood pressure and reactions to adrenalin.

At the time of the last post-castration determination the animals were in excellent health. They romped about when removed from the pens. Nutrition was normal. All, except one, of the animals had gained in weight. Those of series B became excessively fat. In several instances the dogs were in a more

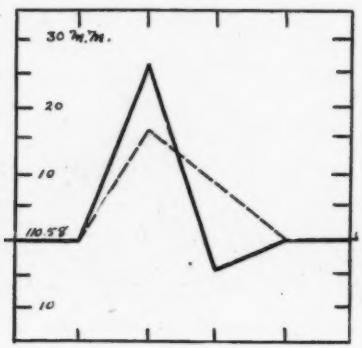

Fig. 3. Composite curves of series A, showing blood pressure responses to standard doses of nicotin. The continuous line represents reactions before; the broken line after castration.

or less complete state of priapism during healing of the wounds in the scrotum.

In five dogs noticeable alterations in disposition were manifest. No. 44 gave much trouble during the first two determinations of blood pressure. It further evidenced signs of aggressiveness by snapping at the attendant while being fed and in tearing out the front of its cage upon two occasions. The dog gradually became less active after castration and gave no further trouble. No. 37 was troublesome during experimental periods but after the removal of the testes became timid and lay as completely con-

cealed from view as possible. It crouched at the approach of the keeper or strangers and did not forcibly resent being handled. No. 66 was a large and active but gentle dog. After castration no particular change was noted in its disposition save an inclination to lie quietly in its kennel. Nos. 104 and 109, both bull dogs, fought continually when put together. About two weeks after castration they were again placed in a large pen with other operated animals and there remained without quarreling. All

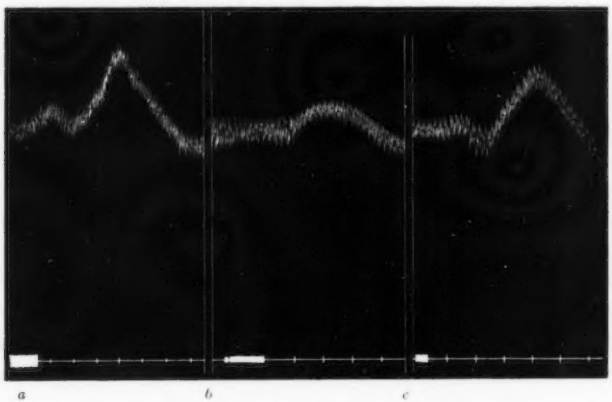

Fig. 4. Reactions of Dog No. 66 to 1 cc., 1:2,000 nicotin. *a*, March 13, 1914, before castration. *b*, April 7, 1914, after the operation and April 10, 1914, after reception of testicular graft placed April 7. Time, five seconds.

of the dogs of series B became fat and less active after removal of their testes.

The lowered activity of these animals may have resulted partially from confinement. However, the greatest changes in disposition were shown by animals with most marked loss of sympathetic irritability.

The well-known dispositional and emotional changes in geldings and steers are characteristic results of gonadectomy. It is also generally accepted that removal of the primary reproductive organs is followed by atrophy of the secondary organs and ulti-

mately by loss of sexual desire. Hatai[5] has recently shown that measurable atrophic changes occur in the brain and spinal cord of white rats deprived of their testes. The changes noted were slight but constant. These changes in secondary sexual signs and somatic alterations demonstrate that the animal's normal economy necessitates the presence of functioning testes. Deprived of these organs structural changes arise followed by alterations in physiological and emotional processes.

The sympathetic nervous system is known to possess important regulatory influence upon the viscera and blood vessels of the body. The work of William James,[6] Lange, and Darwin indicates that impulses arising from the viscera and periphery of the body are interpreted in the form of emotions; they are mental representations of bodily activities. Dispositional characteristics, consequently, are the outward manifestations of emotional conditions. Accordingly, any alteration in the impulses reaching the brain from the viscera or periphery will be shown as modifications of emotional and dispositional states. Such manifestations were noted in my castrated dogs which showed marked loss of sympathetic irritability. Therefore, if the emotions are dependent upon nervous impulses, the dispositional changes noted in castrated dogs are directly referable to the lowered activity of the sympathetic nervous system.

SUMMARY AND CONCLUSIONS

I. Removal of the testes is followed by a slight fall in blood pressure, while,

II. Vaso motor reactions to adrenalin remain unchanged.

III. Blood pressure reactions to nicotin are constantly lowered as a result of gonadectomy, therefore,

IV. The effects of castration are upon the sympathetic nervous system proper.

[5] Hatai, S.:(a) Jour. of Comp. Neurol., 1911, xxi, p. 155. (b) Jour. Exp. Zoöl., 1913, xv, p. 297.

[6] James, William: The Principles of Psychology, Henry Holt and Company. New York, 1890. Reference is made to Lange's and Darwin's works.

V. The lowered irritability of the sympathetic system may partially account for the dispositional changes in castrated dogs.

VI. Incomplete data indicate that irritability of the sympathetic nervous system is increased in castrated dogs by the presence of testicular transplants.

VII. It is, therefore, concluded that a relationship exists between the normal functioning testes and irritability of the sympathetic nervous system proper.

The writer wishes to express his gratitude to Dr. R. G. Hoskins for many valuable suggestions and the personal interest shown in this work.

ON THE CAUSE OF THE PHASE DIFFERENCE FREQUENTLY OBSERVED BETWEEN HOMONYMOUS PEAKS OF THE ELECTROCARDIOGRAM

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If the time of occurrence of the several maxima of the electrocardiogram in the three customary leads is determined with reference to a fixed point of time in the heart cycle, it appears that in general the maxima of homonymous peaks do not occur simultaneously. Attention has been directed to this point by Einthoven (1) and it was made the subject of a special study by Fahr (2) at the Leyden laboratory. If one may judge from the lack of discussion in the literature, the importance of this matter in connection with certain striking peculiarities of the electrocardiogram seems not to have been generally recognized. That the homonymous peaks correspond in a broad sense to the same cardiac process seems quite beyond doubt and the difference of as much as .01 second frequently observed between the times of the maxima in two different leads appears at first sight paradoxical. Of the fact there can be no doubt whatever. To the evidence obtained by Fahr from comparative measurements of simultaneous records of the heart sounds and electrocardiogram, the writer is able to add corroborative data obtained from the simultaneous records of pairs of leads made with two galvanometers. The galvanometers were used in series after a manner suggested by Bull's arrangement of galvanometer and phonoscope (3). One of these galvanometers is essentially a duplicate of the original string galvanometer at Leyden[1] and its constants do not differ greatly from

[1] I wish to take this opportunity of acknowledging my obligation to Professor Einthoven for kindly placing at my disposal drawings and data for the construction of this instrument.

the published values for the Leyden instrument. The second is a smaller instrument, provided however, with a powerful electromagnet and with the same optical system as the larger instrument. Though primarily designed to be used with short strings for high period work, this instrument when equipped with an especially good quartz string[2] 60 mm. long, adjusted to the standard sensitiveness for recording electrocardiograms, gives an aperiodic deflection complete to 99 per cent of the steady value in 0.02 second and the deflections are proportional to the strength of current over the range employed, so that the same lead recorded on either instrument gives measurements which are identical within the usual limitations of the method. As pointed out by Fahr in the paper before mentioned, it is essential in comparing successive records to choose such as are taken in the same phase of respiration. In the present work a pneumograph and tambour were employed to indicate to the operator the phase of respiration and the records were begun in the expiratory phase. Sometimes an inspiration occurred before the record was completed, but the first or the second cycle on the plate was in the expiratory phase.

In figures 1 and 2 are reproduced simultaneous records of leads I and II, and I and III from a normal young man. In both records the lower and smaller line is lead I recorded by the small galvanometer. The magnification is 750 diameters. The heavier curve is lead II in figure 1 and lead III in figure 2 and is the record of the large galvanometer. This was the farther of the two instruments from the camera and, owing to the peculiar optical arrangement, the magnification is considerably greater, about 1250 diameters. The speed of the plates was approximately 100 mm. per second and the vertical lines mark off intervals of 0.01 second. The lack of simultaneity in the maximum points of R_I and R_{II} in figure 1 and between R_I and R_{III} in figure 2 can be readily seen by inspection of the curves. On careful measurement of the negatives under a microscope provided with a micrometer stage, it

[2] For a note on the method of preparing these strings see Proceedings of the American Physical Society, Oct. 18, 1913, Silvered Quartz Fibers of Low Resistance obtained by Cathode Spray, H. B. Williams, Physical Review, Vol. 2, Series 2, p. 402, 1913.

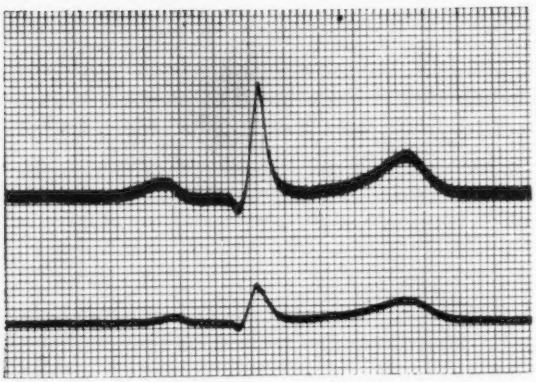

FIG. 1.
Abscissa, 1 division .01 second.
Ordinate, 1 division 10^{-4} volt.
Upper curve, lead II.
Lower curve, lead I.

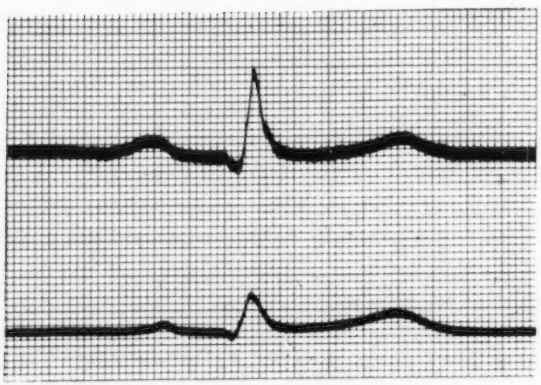

FIG. 2.
Abscissa, 1 division .01 second.
Ordinate, 1 division 10^{-4} volt.
Upper curve, lead III.
Lower curve, lead I.

appears that this difference amounts to .006 second and that the maxima of R_{II} and R_{III} are practically simultaneous and lagging .006 second behind the maximum of R_I. The difference in phase is even more striking in the case of the peaks P, where the maximum is reached more than .02 second earlier in leads II and III than in lead I.

About two years ago Einthoven (4), (5) introduced into the literature of the electrocardiogram his schema of the equilateral triangle and the conceptions of the manifest value of the potential difference in the heart and its direction at any instant. The study of form peculiarities of the electrocardiogram by this method has proved very illuminating and by a graphic application of it the difference in phase above mentioned becomes much more comprehensible. In the accompanying table I, are given the values of

TABLE I

ABSCISSA	e_1	e_2	e_3	E	α
20.0	3.2	4.4	1.2	4.5	45°
*20.8	5.0	13.0	8.0	13.4	68°
21.0	5.0	15.0	10.0	15.3	71°
†21.4	4.6	15.6	11.0	16.7	74°

* Time of maximum of R_I.
† Time of maximum of R_{II} and R_{III}.

the potentials in 10^{-4} volt obtained from measurements of the negatives of figures 1 and 2 for four points of time in the Q–R–S group including the times of maximum of the R peaks. The column headed e_1 gives the values in lead I, etc. The columns headed E and α give the manifest values of the potential difference and the direction in terms of the angle formed with the direction of lead I. The values are calculated from the formulae (7) and (5) of the paper of Einthoven, Fahr and DeWaart (5) above cited, the same reference letters and conventions being employed. In figure 3 the Einthoven triangular schema is drawn and radiating from the center is a series of lines whose directions form angles with the horizontal line equal to the angles, α, in the table and whose lengths are drawn proportional to the manifest values, E.

By the definition of the manifest value of the potential and the angle α, the projection of any one of these lines on any side of the triangle will be proportional to the value e_1, e_2, or e_3 according as the projection is made on the top, left hand or right hand side of the triangle. The second line at the angle 68 degrees corresponds to the time of maximum value of R_I and the longest line at angle 74 degrees to the time of maximum of the peaks R_{II} and R_{III}. The projections of these lines on R–L corresponding to lead I are drawn. One sees at once that the projection of the shorter 68 degree line on this side of the triangle is longer than that of the longer 74 degree line. As the value of the potential is increasing

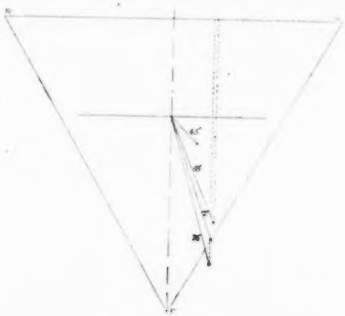

FIG. 3

in scalar magnitude its direction is constantly changing so that while its length and direction were such at the time of abscissa 20.8 as to produce a maximum projection on lead I, the greater length at the time of abscissa 21.4 has been more than offset so far as the projection on lead I is concerned by its rotation toward a direction perpendicular to lead I. We must, therefore, in thinking of the potential fluctuations accompanying cardiac contraction constantly bear in mind that they are *vector quantities*. The rapid change in direction frequently causes more profound alteration in the form of the curve than does the rate of change in scalar magnitude during a given time interval. From the schema it is clear that the 74 degree vector gives a maximum on the sides of the triangle corresponding to leads II and III, and in electrocar-

diograms from normal hearts it is often the case that the maxima of R_{II} and R_{III} are simultaneous and that of R_I is in advance. This is true in one of the reconstructed curves published by Fahr and in the reconstructed curve given in the paper of Einthoven, Fahr and De Waart. However, no general rule can be formulated. Of six normal hearts investigated by the writer, three have had R_I in advance with R_{II} and R_{III} in phase, two have had R_I and R_{II} in phase with R_{III} lagging and in the sixth all three summits were in phase. From this it appears that no statement of probability can be given which will afford much assistance in the analysis of other electrocardiograms and if the peaks are not manifestly in phase some method of measuring the difference will be necessary in order to evaluate the curves.

TABLE II

ABSCISSA	e_1	e_2	e_3	E	α
1	−0.5	0.5	1.0	1.00	120°
2	1.0	−0.5	−1.5	1.54	−49°
3	4.9	0.5	−4.4	5.40	−25°
4	8.2	5.2	−3.0	8.30	9°
5	4.7	−0.3	−5.0	5.60	−33°
6	−0.5	−1.5	−1.0	1.54	−109°

The electrocardiograms of figures 1 and 2 are of relatively simple form. More complicated curves are frequently met, especially in the records of people with diseased hearts. It often happens that the Q–R–S group will be of simple form in two leads and very complex in the other one. At times the peak R is distinctly bifid in one lead while the other two may show no trace of departure from a smooth curve, or there may be a slight thickening or notching of the line. In these more intricate curves the vector diagram is of great assistance to the imagination in picturing the march of events in the cardiac muscle. One set of reconstructed curves published by Fahr (6) illustrates this point very well. The form of the Q–R–S groups of these curves is sketched in figure 4. In table II are given the values of the potentials in the three leads and the manifest values, E, and angles, α, for six points

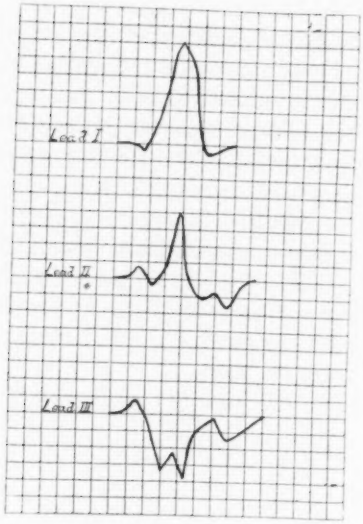

FIG. 4

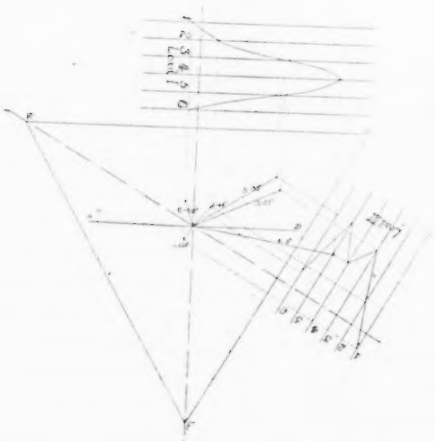

FIG. 5

of these curves. In figure 5, the values of the columns E and α are plotted as vectors in the triangular schema. To project six lines on one side of the triangle would lead to confusion and to avoid this a series of lines has been drawn parallel to the side L–F corresponding to the third lead and another series parallel to the side R–L corresponding to the first lead.

These lines are numbered from one to six and vector 1 is projected on line 1, etc. As before mentioned, the projection of any vector on a side of the triangle (or on a line parallel to a side), is proportional to the value e_1, e_2 or e_3 which would be recorded in the corresponding lead at the instant whose potential the vector in question represents. If, then, we project the vectors in order on one of the series of parallel lines and draw a curve through the points thus marked off on them, we obtain, as shown, a curve whose form is seen to be the same as that of the original curve in the corresponding lead. In figure 5 the construction has been made for leads I and III. In order to see the constructed curve in the proper relation the figure must be turned so that the legends "Lead I," "Lead III" are seen right side up. It is most instructive to observe the manner in which the projection of the same series of vectors produces in lead I a smooth curve and in lead III a complex curve with two minima. Reference to the column E in table II shows that the scalar values of the potential increase regularly to a maximum and then diminish. In this case the peculiar form of the curve in lead III is due entirely to the manner in which the *direction* of the potential is shifting.

We can affirm with considerable confidence that the form of the electrocardiogram is a function of the path pursued by the wave of activity which spreads through the heart during a contraction. That it is a function of other variables is also true, for example, we may cite the effects of the movements of respiration. Its cardinal characteristics are doubtless correctly referred to the mode of propagation of the disturbances which as a whole constitute a contraction of the muscle. If we are ever able to find the explanation for the rapid shifting of the direction of the potential in such cases as the foregoing we may expect to find it involved in peculiarities of the path of propagation or in the relative rates of propa-

gation of the contraction wave along various parts of the path. In certain cases of cardiac disease anomalous notched and doubled curves may appear in the electrocardiogram during a temporary accentuation of the disease to disappear again after a period of rest with resulting improvement in the condition of the heart. This observation lends color to the suggestion that such effects may be an expression of disturbances in the conduction system of the heart. It is well known that profound changes in conductivity may occur without demonstrable anatomical lesions and that it is not infrequent to see complete recovery from conditions which are easily recognized as involving the conduction system of the heart. A study of the electrocardiogram in disturbances of conduction where anatomical lesions can be subsequently demonstrated, in conjunction with the study of the effect of experimental lesions of the branches of this system beyond the primary divisions may be expected to add much to our knowledge of the train of events which make up the cardiac systole.

SUMMARY

Simultaneous records of two leads of the electrocardiogram confirm the conclusion already reached by Einthoven and by Fahr that homonymous peaks are not always in phase. The difference in phase is shown to depend upon the vectorial character of the potential changes in the heart.

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(6) Fahr, loc. cit., p. 163.

STUDIES ON BLOOD AND URINARY AMYLASE

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In 1863 Conheim (1) discovered that the urine of man contains a substance which converts starch into sugar. Two years later Béchamp (2) reported that the urine contains a starch splitting substance, secreted by the cells of the kidney, which he called nephrozymase. In 1886 Holovtschiner (3) demonstrated an amylase in the urine which he thought identical with ptyalin. Breusing (4) in 1887 confirmed the presence of an amylase, but found no reducing sugar after its action on starch. Gehrig (5) at about the same time found that the urine of the dog contains amylase, but in smaller quantities than that of man. Work of this type has been gradually extended, so that at present we know that the urine of most of the warm blooded, and of many of the cold blooded animals contains amylase.

The more recent workers—Wohlgemuth (6), Hirata (7), Corbett (8), and Brown (9), have in the main confirmed the work of the earlier investigators, and in addition Wohlgemuth has greatly improved the methods for quantitative estimation. Most of the recent work has been done with the view of testing the functional capacity of the kidney, and of diagnosing pancreatic lesions. Attempts have also been made to associate variations in the quantity of urinary amylase with various other disorders but without marked success.

It is not surprising that amylase elimination has not been more generally adopted as a diagnostic measure, if we take into consideration that the physiological conditions governing the elimination of these enzymes, as well as their actual source are not definitely known.

The work reported in this paper was undertaken for the purpose of studying further the elimination of amylase in the urine, in order to throw more light on its source as well as physiological significance.

In our attempt to accomplish this end we have collected data from five different lines of investigation: we have studied the daily amylase content of the urine of a number of animals of different species, under conditions as constant, and near normal and natural as the laboratory would allow; we have studied the amylase output in the urine of various species under controlled variable conditions, all the conditions being kept within normal limits; numerous experiments have been carried out on animals with definite anatomical lesions; the elimination of foreign amylase from the blood after intravenous injection has been studied; and finally, numerous miscellaneous experiments with materials and animals selected at random.

METHODS

We have found the iodine color methods best adapted to the conditions under which we have worked, for the reason that frequently with urine samples after prolonged incubation with starch, no reducing sugar is demonstrable, consequently the estimation of reducing sugars would not be applicable. In 1881 Roberts (10) published a method for the quantitative estimation of amylase. He measured out a definite volume of starch solution, incubated it with a definite volume of enzyme solution and determined the length of time required to carry the hydrolysis to the achroodextrin stage. This method is based on the assumption that the enzyme concentration bears a simple inverse ratio to the length of time required to carry the hydrolysis to a definite point. This assumption has since that time been proved erroneous, for high concentrations. We have convinced ourselves by numerous tests, that when the dilution of the enzyme is high, such as we find in the urine and sera of most animals, the time required to carry the hydrolysis to a definite point is a reliable criterion from which we can judge the relative quantity of amylase present.

Recently Wohlgemuth (6) has devised a method which has found ready acceptance and appears to be very reliable. He prepares a series of tubes, putting an equal volume of 0.1 per cent starch into each and keeping them all in ice water. He then adds successively increasing amounts of enzyme to the tubes, puts them into a water bath kept at from 38°–40°, for a definite length of time, thirty minutes in the short method, and twenty-four hours in long method, then removes all the tubes at the same time, adds an equal amount of $\frac{N}{10}$ iodine, dilutes and compares colors. The last tube showing the starch-iodine blue is taken as the "limes," and the D calculated. The D represents the number of cubic centimeters of 0.1 per cent starch carried to the endpoint by one cubic centimeter of the enzyme in the incubation time. Some workers have modified this method slightly, such as using a different concentration of starch, different incubation time, and the achromic point as the end. The principle in these cases is not modified.

Throughout our work we have used a method similar to that of Roberts but have used the nomenclature of Wohlgemuth. In much of the work both methods were employed using them as checks on each other. We have used a 1 per cent starch solution exclusively,—Merck's soluble starch, using 0.9 per cent NaCl as the solvent and dilutant. Two volumes of urine were incubated with one of starch, usually 10 cc. of urine to 5 cc. of starch, care being taken to warm both to the incubation temperature before mixing. In case of serum we used one volume of serum to five of starch. 38°–40°C. was found to be the most favorable temperature and was maintained as the standard throughout all the work. The incubation was carried to the point where no red color could be detected when a drop of the mixture was added to 1 cc. of dilute ($\frac{N}{20}$) iodine. The time consumed in carrying the hydrolysis to the achroodextrin stage, and the volumes of starch and enzyme solution being known, the D value was calculated. The D means the number of cubic centimeters of a 1 per cent starch solution hydrolysed to the achroodextrin stage in one hour.

Neither method is free from criticism. Roberts' method is based upon an assumption which does not hold true for highly concentrated enzyme solutions. In the use of Wohlgemuth's method it is sometimes necessary to change the dilutions or the incubation time in order to obtain a readable series. The urines and sera, except in extraordinary cases, are what we might term highly diluted enzyme solutions, and we find that at these dilutions the activity is inversely proportional to the dilution. Because of this fact, because of the agreement of results with those obtained by using Wohlgemuth's method, and because fewer tubes and less volumetric work are required, we have employed the Roberts method.

The urine employed in the experiments was kept as free from contamination as laboratory conditions would allow. In the case of laboratory animals, they were kept in clean metabolism cages and the urine collected in clean bottles which had previously been rinsed out with an alcoholic solution of thymol. It has been suggested that thymol inhibits to some extent the activity of amylase, but we have satisfied ourselves that the mischief due to the thymol is less than that due to decomposition, consequently we have made it a part of our routine. Urines contaminated with liquid foods, vomitius, feces, or which were badly decomposed were thrown out.

Serum was obtained by centrifuging freshly drawn defibrinated blood. In a few cases the blood was drawn and allowed to clot and the serum squeezed out. It is known that fibrin adsorbs some of the proteolytic enzymes and it was suggested that some amylase might be lost in this way. We have carried out experiments in which we hirudinized part of a sample and defibrinated the other. The plasma and serum thus obtained showed no difference in amylase content. Consequently we have concluded that the serum obtained from defibrinated blood contains all the amylase which is in solution in the blood at the time it is drawn.

EXPERIMENTAL RESULTS

1. Comparative work on animals under constant normal conditions

With the exception of man these animals were kept in cages as described above, and given all the water they would drink, and a mixed diet consisting of bread and meat scraps from the University Commons. The samples of human urine were obtained from men working in the laboratory and from all appearances healthy. No particular attention was paid to drink or food. When obtainable, tests were made only on mixed

TABLE I

A table of urine and serum averages for dogs kept under the conditions described above; urine averages for five successive days

DOG NO.	WEIGHT	URINE			TOTAL UNITS IN 24 HRS.	D. PER KILO	SERUM D.	REMARKS
		VOL. OF URINE IN 24 HRS.	Sp. Gr.	D				
	kgm.	*cc.*						
3	6.4	173	1034	0.20	34.60	5.4	7.5	
							7.5	3 days later
5	5.8	357	1023	0.07	24.99	4.3	6.2	
							5.6	10 days later
6	9.0	350	1030	0.06	21.00	2.3	5.6	
							5.0	10 days later
7	7.0	220	1021	0.04	8.80	1.2	6.8	
14	6.5	220	1027	0.23	50.60	7.7		
20	8.0	206	1020	0.03	6.18	0.7	7.8	
21	9.1	395	1030	0.10	39.50	4.3		
22	15.3	770	1010	0.20	154.00	10.0		
23	12.4	525	1010	0.05	26.25	2.1		
24	6.4	126	1023	0.12	15.12	2.3	5.0	
25	8.3	190	1035	0.08	15.20	1.8		
26	7.3	120	1038	0.15	18.00	2.4		
27	5.5	150	1040	0.03	4.50	0.8	5.0	
29	7.4	250	1032	0.34	85.00	11.4		
Averages	8.0	289	1027	0.12	35.83	4.0	6.2	
4*	7.1	182	1027	1.52	276.64	38.9	7.5	
							7.0	3 days later

* A dog apparently normal. We have found one other. It is so exceptional that we have left him out of the list in calculating the averages.

TABLE II

Serum and urine D averages in normal cats

CAT NO.	WEIGHT	VOL. OF URINE	URINE		TOTAL UNITS	UNITS PER KILO	SERUM D
			Sp. Gr.	D			
	kgm.	*cc.*					
2	3.6	42	1045	0.56	20.0	5.5	7.5
3	3.5	60	1042	0.65	37.6	10.7	7.5
20	2.9	55	1052	0.21	10.3	3.5	6.0
21	3.4	60	1056	0.20	11.7	3.4	7.5
22	3.5	40	1052	0.20	8.0	2.4	6.6
23	3.5	100	1032	0.12	12.0	3.4	6.0
Averages	3.0	59	1046	0.33	16.6	4.8	6.8

TABLE III

Five day urine D averages in three normal men

INDIVIDUAL	WEIGHT	VOL. OF URINE	SP. GR.	D	TOTAL	UNITS PER KILO
	kgm.	*cc.*				
Mr. C.	74	1002	1027	2.11	2117	28.8
Mr. W.	68	973	1020	1.21	1204	17.7
Mr. K.	60	1004	1024	1.28	1238	20.6
Average	67	993	1023	1.53	1520	22.3

TABLE IV

This table summarizes the results on a normal pig, kept in a cage for 8 hours during which time four samples of urine were obtained. The volume, total units, and units per kilo in the average space are calculated for 24 hours. Weight of animal 61 kg.

SAMPLE	VOLUME	SP. GR.	D	TOTAL	UNITS PER KILO	SERUM D
	cc.					
1	110	1025	1.65	181.5	2.9	
2	90	1025	1.67	150.6	2.4	
3	120	1023	1.20	144.0	2.3	
4	40	1023	1.35	52.0	0.8	
Average	1080	1024	1.46	1584.3	25.9	6.6

TABLE V

Amylase content of urine, pancreas, and serum of kittens at various ages

AGE	URINE D	SERUM D	PANCREAS EXTRACT D
6 days	0.017	0.2	trace
16 days	0.071	1.39	0.71
6 weeks	0.025	6.00	3.50
7 weeks	0.10	6.60	3.30

twenty-four hour samples. Care was taken to record the volume of urine, the specific gravity, the D value, the total units in the twenty-four hour samples, and the units eliminated per kilo body weight. The "total units" is obtained by multiplying the D value by the figure representing the twenty-four hour volume.

In addition to the work on animals already tabulated, experiments were carried out on the goat, raccoon, woodchuck, rabbit, pups of various ages, and the opossum.

We have detailed the foregoing tables because they present points of special interest Most of our later work was done with materials from man, dogs, and cats, consequently detailed knowledge of what might be considered normal in these animals is of vital importance. We have tabulated the results on the pig because of their close resemblance to those from man. The table of results on kittens shows that the blood and urine of a six weeks old kitten approximate that of an adult cat in amylase content, and suggests strongly that the pancreas is in a large measure responsible for this.

We shall not make detailed tables of the work on other normal animals, for we feel that we can avoid unnecessary repetition, and add greater clearness by putting the conclusive findings on these animals into a general summarized table of results on all the normal animals studied (table VI).

TABLE VI

Results on normal animals summarized

ANIMAL	D OF URINE	D OF SERUM	D PER KILO WT.
Man	1.53		22.38
Dog	0.12	6.2	4.00
Cat	0.33	6.8	4.80
Pig	1.46	6.6	25.96
Rabbit	1.05	2.5	33.51
Woodchuck	9.00	18.0	131.00
Raccoon	0.34	20.0	7.03
Goat	0.013	0.53	0.14

2. Results on animals under variable normal conditions

In the first part of our record of results we have given tables in order to show in detail the various factors which might bear some relation to the amylolytic strength of the urine. It appears that for comparative work on average normal animals as well as for the work of part 2 the most significant figure is that representing the units of amylase eliminated per kilo body weight. The purpose of the experiments reported under part 2 is to compare the amylase output from animals, under what we might term extreme normal conditions, with those under average normal conditions, consequently we feel that it is necessary to record only the units of amylase eliminated in the urine per kilo body weight.

(*a*) *Diuresis.* These experiments were done on men and cats. Normals were taken for five successive days during which time no special attention was given to the amount of water taken in. On the five succeeding days the amount of water taken was approximately doubled. In case of the cats it was given by stomach tube. The units of amylase per kilo body weight eliminated in the urine by Mr. C. during the diuresis period were 38.01 as compared with 28.3 under average normal conditions. In case of Mr. K. the units during diuresis were 37.46 as compared with 20.6 as average normal. The cats gave 4.8 units as normal—an average for two cats, and 6.9 during diuresis.

(*b*) *Water starvation.* In these experments the animals were deprived of water so long as no signs of extreme suffering appeared. The goat was water starved for eight days and seemed none the worse, cats for three days, and man one and one-half days. In all cases the specific gravity rose considerably above the average normal. In case of the goat it rose from 1045 to 1065 during the first three days and remained at the latter figure to the end of the eighth day. In man (Mr. C. and Mr. K.) the average per kilo weight during water starvation was 18.2 units and under average normal conditions 24.5 units The average of the cats, urine was 4 units per kilo weight during the time the water was withheld, as against 4.8 units under average normal conditions.

The urine of the goat normally contains scarcely more than a trace of amylase, and during the starvation period, the urine was not passed every day. There was no marked change in the amylase content of the urine of this animal.

(*c*) *Feeding $NaHCO_3$.* Cats were used exclusively. The point to be determined was whether by rendering the urine of this animal less acid, the amylolytic activity would be increased. We found 5 grams of $NaHCO_3$ the dose per day necessary to maintain the urine approximately neutral. Most of the cats developed indigestion with vomiting and diarrhea, thus making some experiments incomplete. In one cat no complications arose and the experiment was carried to the point planned. In this cat the normal amylolytic strength of the urine per kilo weight was 15.6 units—considerably above that of the average cat. During the period of $NaHCO_3$ administration the cat averaged 3.1 units per kilo. Averages could not be obtained from the other cats for the reasons already stated, but the results obtained showed a decrease after the administration of $NaHCO_3$ was begun.

(*d*) *Feeding of starch.* Both cats and dogs were used, and the starch given daily by stomach tube, 100 cc. of 2 per cent starch to the cats and 300 cc. to the dogs. There was no significant change in the amylolytic strength of the urines of any of these animals.

(*e*) *Feeding amylase.* These experiments were done on animals secreting no salivary amylase. The point in mind was to establish or disprove the probability of urinary amylase being in part ptyalin reabsorbed from the digestive tract. Dogs and cats were used. No change was demonstrable in the case of dogs, although one animal received 100,000 units per mouth for four successive days. One cat which received 9,000 units for four days showed no increase in the urine. Two cats, each receiving 17,500 units for four days showed increases. One cat eliminated 8.2 units per kilo weight during the feeding against 1.9 units as normal. The other cat eliminated 27.4 units against 4.7 while normal.

(*f*) *Injection of amylase into the intestine.* One dog was used for this work. The animal was anasthetized with ether and a small median abdominal incision made. A loop of the jejunum was pulled up and 100,000 units injected through a needle. The wound was closed by suture and healed without complication. The animal under normal conditions never eliminated more than a trace of amylase. During the four days immediately following this injection he averaged 3.3 units per kilo weight, the highest being on the second day where 7.3 units per kilo were eliminated in the urine. The serum showed no definite change in amylolytic power.

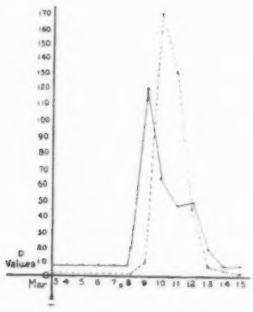
3. Results on animals with definite anatomical lesions

(*a*) *Ligation of pancreatic ducts.* Two dogs were used for this work. The animals were kept in metabolism cages and the normal curve of the amylolytic activity of the urine determined. The animals were then anesthetized, a median abdominal incision made and the two main ducts of the pancreas firmly ligated. The wound was closed by suture and in all cases healed without complications. No marked digestive disturbances were noted, the animals being fed largely on lean meat. At the end of the experiments the animals were killed. The autopsy revealed that the ligatures had disappeared and that the ducts were draining the juice from the pancreas into the gut. Both dogs gave similar results. The curve below (fig. 1) is that of dog III, showing the relative D's of the urine and serum on successive days of the experiment.

(*b*) *Trauma of the pancreas.* Wohlgemuth and Noguchi (11) have reported that in case of abdominal trauma followed by symptons which point toward injury to the pancreas, a quantitative amylase test on the urine may be of value in clinching the diagnosis. They find that in traumatic injuries to the pancreas the amylolytic activity of the urine is increased. We carried out one experiment for the purpose of determining this point. A dog was used for the work. After determining the normal output per kilo weight, the animal was anesthetized, a median

abdominal incision made, and the pancreas manipulated and crushed between the fingers. The wound was sutured and healed by first intention. The normal output per kilo weight was 3 units, and the average output for the nine days following the operation was 10 units. The animal was killed on the ninth day and the pancreas was found reddened and swollen with a few small patches of fat necrosis. No other abnormalities were found.

(*c*) *Removal of the pancreas.* Schlesinger (12) has reported that pancreatectomy leads to a complete disappearance of amylase from the blood. Other observers claim a reduction but not a complete disappearance. This condition should lead to a decrease in the urine. In order to determine the facts, four dogs



——— Serum.
- - - - Urine.
* Pancreatic ducts ligated Mar. 7.

FIG. I. CURVE OF DOG III.

and two cats were pancreatectomised, and also one woodchuck. The dogs behaved as the average pancreatectomised dog, but the woodchuck died during the first day. In this animal the pancreas is so scattered that its removal without serious injury to the adjacent viscera is almost impossible. The normal output per kilo in dog C was 12.8 units, and after pancreatectomy 1.5 units. The serum of this animal tested 7.5 units before and averaged 2.5 units after the operation. The urine of dog 22 averaged 7.7 units before and a trace after operation—the serum testing 6.8 units before and 2.1 units after. Dog 23 averaged

3.5 units in the urine per kilo before, and a trace after the operation,—the serum testing 6 units before and 3 units after. Dog 25 averaged 4.8 units in the urine per kilo before and 0.2 unit after operation—the serum 7 units before and 2.1 units after.

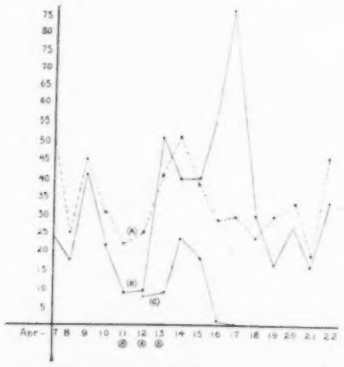
The cats gave results very similar in nature, averaging 4.6 units per kilo in the urine before, and 0.33 units after operation. The sera of these animals were not tested.

Serum and urine was also obtained from one pig which had been pancreatectomised nine weeks before, and which ran a typical pancreatic diabetic sugar curve. The autopsy showed the operation to have been complete. The urine contained only a trace of amylase, and the serum tested 2.4 units.

(*d*) *Phloridzin poisoning.* Phloridzin renders the kidney more permeable to sugar, but Sollman (13) has shown that the permeability is not increased for all substances in the urine. Two series of experiments were done on dogs in order to determine whether there is an increased elimination of amylase in the urine parallel with the increase in sugar. One experiment was completed without any disturbing complications. In another dog the experiment was interrupted by vomiting and diarrhea, but the results to that point agreed in essentials with those from the dog in which no complications arose. In both animals the amylase output during the diabetic period was approximately double that of the normal period. The following graphs were constructed on basis of figures obtained from the dog which developed no complications and show the total amylase units, grams of sugar in the urine, and the daily volume of urine (fig. II).

(*e*) *Removal of the kidneys.* These experiments were done in order to determine whether the serum increases in amylolytic activity with the kidneys completely eliminated. Two dogs were so operated and lived 26 and 31 hours respectively after the operation. These animals were considerably depressed during the time of survival. The sera showed some fluctuations but no constant rise. We do not consider these experiments as entirely conclusive, but the results agree in general with those obtained by Van de Erve (14) who found that ligation of the renal arteries does not appreciably alter the concentration of serum amylase.

(*f*) *Injection of tartaric acid.* Underhill (15), Underhill, Wells and Goldschmidt (16) have found that subcutaneous injection of tartrate leads to extensive destruction of the epithelium of the proximal convoluted tubules in the kidney. Other observers (Wohlgemuth (17) Corbett (8)) have found that in nephritis the amylase in the urine is less than normal. It should therefore be possible to determine whether the amylase passes through the proximal convoluted tubule or elsewhere. If this were found to be the case, then the quantitative amylase test might not only be of value in the diagnosis of nephritis, but would also



⊕ Injection of 3 gm. phloridzin intravenously.
(a) Urine volume in 24 hrs. divided by 10.
(b) Total units of amylase in urine in 24 hrs.
(c) Sugar in urine in grams each 24 hrs.

FIG. II. DOG VI—INJECTION OF PHLORIDZIN.

aid in determining what structure of the kidney is most seriously involved. We are at present engaged in such experiments, and although not nearly completed, the results so far obtained indicate that in tartrate nephritis the amylase elimination runs closely parallel with the rate of secretion of urine. We have found however in one case, with the kidneys so injured that they eliminated during the first hour only 12 per cent of a quantity of phenolsulphonephthalein, a normal output of amylase.

(*g*) *Parathyroid tetany and severe depression.* Early in our work we made tests on urines obtained from animals dying dur-

ing an attack of parathyroid tetany, and found that in some cases the urine of such animals contained a relatively high concentration of amylase. We then undertook a systematic investigation on animals in parathyroid tetany. Eleven dogs and seven cats were used. Of this group nine dogs and six cats developed tetany, the others remaining normal, perhaps because of incomplete parathyroidectomy. We found that in animals which remained apparently normal until the onset of the fatal attack, the amylase content was practically normal, but that animals dying after prolonged and severe tetany eliminated urines of relatively high amylolytic power, and that the urines of animals dying after a prolonged period of depression, or a combination of tetany and depression constantly contained a larger amount of amylase. We were thus led to suspect that profound tissue depression is more constantly associated with an increase in urinary amylase than parathyroid tetany. Consequently we made a large number of tests on urines obtained from animals dying after prolonged profound depression from other causes. We found these urines uniformly of high amylase content. The following table (table VII) is a summary of the results of these experiments. The results recorded are those obtained from the last sample of urine obtained from the living animal, or taken from the bladder shortly after the death of the animal. In most cases we were not able to obtain fresh blood, consequently only a few of the serum tests are recorded.

4. Intravenous injection of foreign amylase

We next undertook to determine what portion of and how rapidly foreign amylase when injected intravenously into dogs is eliminated in the urine, and also to determine how rapidly the enzyme disappears from the blood. Two types of experiments were carried out, one with dogs in metabolism cages, and the other crucial experiments on dogs under ether anesthesia.

(*a*) *Dogs in metabolism cages.* The animals were given the same volume of water, 250 cc. each day by stomach tube, and fed on a constant diet. The total units of amylase in the urine

were estimated each day and an average calculated for a period of six days. A definite volume of human saliva of known amylolytic strength was then injected intravenously and the total units appearing in the urine each succeeding day determined until the animal reached the normal level. The sera of these animals

TABLE VII

D values of urines and sera obtained from animals dying in parathyroid tetany, profound depression, or a combination of both

ANIMAL	D OF URINE	D OF SERUM	REMARKS
Dog.....	0.08		Died during first attack
Dog.....	0.12		Died during first attack
Dog.....	0.06		No depression—died during second attack
Cat.....	0.21		Moderate tetany until last attack
Dog.....	1.00	6.4	Severe tetany for two days. Active between attacks.
Dog.....	6.60		Depressed for two days
Dog.....	7.50	16.5	Depressed for two days
Dog.....	12.00	6.6	Depressed for 36 hours
Dog.....	5.00		Severe tetany for 3 days
Dog.....	1.10		Died on second day in violent tetany. Depressed during last day
Cat.....	7.5	7.5	2 days without tetany
Cat.....	3.5		Violent tetany and depression 2 days
Cat.....	15.00		Depressed 4 days—found dead
Cat.....	3.00		Depressed 2 days
Cat.....	1.5		Moderate tetany for 3 days, last day depressed
Dog.....	1.00		Died of pneumonia in profound depression for 2 days
Dog.....	10.00		Hypophysis removed 17 days before. Great depression 3 days
Dog.....	5.40		Died of septicemia—depressed 2 days
Dog.....	3.10		Died of infection after being bitten by another dog

were also tested and it was uniformly found that by the end of the first twenty-four hours after injection, the serum had returned to the normal level. Eight such experiments have been completed with uniform results. On account of the uniformity of the results we shall give the figures for only one of the most typical animals.

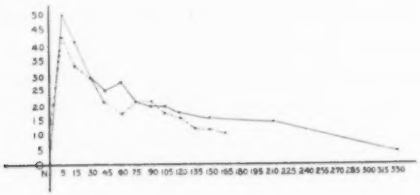
This animal never under normal conditions eliminated more than a trace of amylase in the urine. He was given 6,250 units intravenously. During the first day after the injection 875 units appeared in the urine, and during the second day 672 units. On the third day and the days following there was a mere trace. In this animal about one-fourth of the amount injected was accounted for in the urine, in some animals only one-tenth. The increase lasted two days. In several cases there was still an increased amount on the third day and in one case where only 1,500 units were injected the increase in the urine was confined to the first day.

In only one case did an animal die of septicemia after the injection of saliva. On the whole dogs tolerate human saliva better than solutions of Holadin, taka-diastase, or extract of dog's pancreas. A dog on an average will tolerate about 2.5 cc. of human saliva intravenously per kilo body weight. When the dose is much in excess of this the animal may show signs of collapse resembling anaphylactic shock.

(*b*) *Crucial experiments.* These experiments were done for the purpose of determining the rate of disappearance of foreign amylase from the blood. The dogs were kept under ether anesthesia, and a vein and an artery prepared in the usual way for injection and bleeding. A normal sample of blood was drawn, then a volume of saliva of known amylolytic strength injected. In five minutes another sample of blood was drawn, and thereafter at intervals of fifteen in some, and thirty minutes in other animals, until death. As much 0.9 per cent NaCl was injected each time as blood drawn. The serum was obtained from the blood in the manner already described. The amylolytic power of these sera was then tested. The urine was also collected but unless diuretics were used the volume obtained was usually very small. Quantitative tests for amylase were made on the samples obtained.

In one series of experiments the kidneys were left intact, in another the renal arteries were ligated. We found no characteristic difference between the curves of disappearance of amylase from the blood in these cases. Caffeine diuresis was also

tried on animals with intact kidneys. It was found that in caffeine diuresis the amylolytic power of the urine, after injection of amylase into the blood rises parallel with the volume of urine secreted. Figure III is composed of graphs showing the relative rates of disappearance of amylase from the blood of animals with intact kidneys, and animals with the renal vessels ligated.



----- Renal arteries ligated. Dog VIII. 7500 units.
—— Normal dog. Dog I (a). 9000 units injected.
N=Normal.
Figures at bottom = min. after injection.
Figures at side = units of Amylase.

FIG. III.

5. *Miscellaneous experiments*

During the course of our work many check experiments became necessary. The results obtained frequently suggested other lines of investigation which in some cases were followed out.

(*a*) *Relation of acidity of the urine to its amylolytic activity.* It is well known that low concentrations of acid inhibit the action of amylase, and that high concentrations destroy the enzyme. It has been suggested that the kidney eliminates a much larger quantity than is detectable in the urine, and that the decrease in activity may be due to the presence of inhibitory substances. The acids have been suspected of being responsible. This could not be universally true for the urine of the goat, which contained the least amylase of any tested, was constantly alkaline. However, the suggestion that in those animals which void an acid urine, the acid may exert an inhibitory influence is not unreasonable.

We attacked this question by determining the H ion concentration of the urine and correlating the findings with the activity, and also attempted to discover a definite relation between titratable acidity and amylolytic activity. We could find no definite constant relation. We also compared the activity of normal urine with neutralized specimens of the same sample and found a decrease in activity as often as an increase, and frequently no change. We used both Na_2CO_3 and NaOH for the neutralization.

(*b*) *Inhibitory substances in the urine.* Two dilutions of saliva, one in which 5 cc. of saliva was diluted to 50 cc. with 0.9 per cent NaCl, and the other 5 cc. of the same sample of saliva diluted to 50 cc. with dog's urine having only a trace of amylase, were tested simultaneously and found to posess approximately the same activity. The experiment was done with eight different samples of urine with uniform results. In case urine was used which had undergone ammoniacal decomposition the activity was decreased. We attribute this to the presence of the ammonia for we found that if enough ammonia is added to fresh urine to give the same reaction and odor as that of decomposed urine and this mixture used as the dilutant, the activity is decreased.

(*c*) *Reduction tests.* We found that frequently when the achroodextrin stage is reached in the action of urinary amylase on starch no reducing sugar is demonstrable with Fehling's solution. This is also true in the case of serum. After the injection of saliva reducing sugar appears in the case of the action of both the serum and urine on starch.

(*d*) *Specific gravity and amylolytic activity.* We found that for a given animal, within limits which may be termed normal there is no direct constant relation between the specific gravity and amylolytic activity. It is not uncommon to find a urine with a specific gravity of 1020 to be as active or more so than a sample from the same animal with a specific gravity of 1027. We also found that when the specific gravity in man falls below 1018 there is always a corresponding decrease in activity. Very frequently on the other hand a high specific gravity is associated with a comparatively low activity. We should recall that in

water starvation the amylase output is decreased, but the specific gravity of the urine is always high.

(e) *Injection of starch.* The results recently obtained by Abderhalden (18) and his collaborators would indicate that serum amylase may be classed with the protective enzymes. He goes so far as to claim that amylase is not normally present, but that it appears after the parenteral administration of starch. We have found amylase present in all the sera examined, although, as stated above, we have not always been able to demonstrate reducing sugar after incubating the serum with starch.

Two experiments were done, using dogs, for the purpose of determining the effect of intravenous injection of starch upon the amount of amylase eliminated in the urine. In both cases there was an enormous increase of amylase in the urine reaching the maximum on the second day after the injection, and lasting about four days.

Numerous other experiments were carried out in the course of this work, largely supplementary to those already described, which will be referred to in the discussion of results.

DISCUSSION OF RESULTS

1. Urinary amylase and diet in adults

Pawlow (39) has described what he considers an adaptation to diet in case of the gastric juice. He finds that the proteolytic power of this secretion depends in part at least, upon the stimulus causing the secretion. The same is asserted of the pancreatic juice. If these facts are to be interpreted on the grounds of more enzyme production on part of the digestive glands, it would not be unreasonable to expect an increase in the blood and urine enzymes,—assuming that these enzymes are in part at least reabsorbed either from the gland or from the digestive canal. We are interested in this primarily because in all the animals we have studied the pancreas is an amylase secreting gland, consequently we might expect that diet would affect the concentration of amylase in the blood and urine.

A number of years ago Nielson and Terry (20) reported that in case of the dog, whose saliva contains at the most only a trace of amylase, after feeding on a high carbohydrate diet the saliva became strongly amylolytic. Mendel and Underhill (21) and Carlson and Ryan (22) have failed to confirm the results of Nielson and Terry, at least their interpretation.

In the work done on this point animals were fed for a relatively short time on a larger amount of carbohydrates than was usual for the animal to take. We carried out a few experiments of this type in which we gave the starch by stomach tube, with negative results. We doubt the utility of such experiments, for several reasons. In the first place the question of specific and constant adaptation of digestive secretions to the variety of food in any instance is still an open one, and in the second place we doubt whether any adaptation requiring such a high degree of specialization on part of the gland could be brought about in any animal by a few weeks or even months feeding on an abnormal diet.

On the other hand, if there is any direct biological relation between the stimulus and the concentration of the enzyme produced as the result of this stimulus, we should expect to find it as a species characteristic rather than an individual one. We should expect in the herbivora and omnivora the amount of amylase produced to be greater than in the carnivora.

The results of the comparative work on normal animals do not entirely support this latter view. The goat is the notable exception. This animal, which is entirely herbivorous, eliminates in the urine scarcely more than a trace of amylase, on an average less than the dog and cat which are naturally carnivorous. The serum of the goat has only about one-twelfth the amylolytic activity of that of either the dog or cat.

The question naturally arises to what extent the amount of amylase in the urine is an index of the amount produced in the body. We have shown that the amylase introduced intravenously does not all reappear in the urine, but that an increase in the blood is followed by an increase in the urine. If it can then be proved that an increased production leads to an increased amount in

the blood, it would be entirely logical to conclude that an increase in production would lead to an increased amount in the urine.

Several facts have been brought forward which may be interpreted as indicating definite relations between the amount of amylase produced and that in the urine. In case of the cats fed on $NaHCO_3$ the amount of amylase in the urine fell considerably below normal. According to Starling (23) alkalis decrease the activity of the pancreas. Hawk (24) also maintains that the intake of large quantities of water leads to an increased activity on part of the pancreas which may in part account for the increase in urinary amylase in water diuresis. A glance at table VI will reveal the fact that the amylase output per kilo for man, the pig, and the rabbit is relatively high compared with that of the dog, cat, and goat. It is known that the saliva of the first three animals contains amylase and that of the last three not. We have shown that removal of the pancreas leads to almost total disappearance of amylase from the urine, and furthermore it has been observed clinically that in diseases of the pancreas depressing its activity, the amylase of the urine decreases. On the whole we feel justified in assuming that in a given animal an increase in the amount of amylase produced leads to an increase in the urine.

Turning again to the results obtained from the goat, we are forced to the conclusion that the low amylase content of the blood and urine signifies one of two things: either this animal produces a comparatively small amount; or the mechanism of destruction is unusually active. We have done no work on the sheep but the results obtained by other workers indicate a similar condition in this animal. Neither of these animals produces a salivary amylase, and both have practically the same feeding habits.

On basis of feeding habits alone we should then expect the rabbit to approximate the sheep and goat in amylase output. Our results show the rabbit to be classed with man and the pig. Besides certain differences in the arrangement of the alimentary tract in the rabbit and the goat, the rabbit's saliva contains an abundance of amylase while that of the goat contains none.

This may in part account for the difference, and also suggests that the urinary amylase is in part reabsorbed salivary amylase.

The dog and cat, both carnivora, show a marked similarity in amylase output.

The omnivora show uniformly a higher amylase output than any of the other animals. Under this class we include man, the pig, the racoon, and possibly the woodchuck. We are applying the term omnivorous to those animals which will eat both meats and vegetable foods regardless of their usual habits. The woodchuck subsists largely on vegetable foods but occasionally kills and eats fowl and small animals. The racoon eats grain when he can get it, but will also kill and eat fowl and smaller animals. We do not know whether the saliva of the woodchuck contains amylase or not, but that of the racoon does not, at least only a trace. The other omnivora, man and the pig both secrete an amylolytic saliva.[1] It should furthermore be noted that the output per kilo in case of the racoon is much lower than that of any of the others in his class. At present we have no explanation to offer for the high amylase output of omnivora, excepting the suggestion that the salivary amylase is a large contributing factor, which would also explain the lower output in the racoon.

Our results point to the following conclusions: Animals feeding on a high carbohydrate diet do not invariably eliminate a large amount of amylase in the urine; feeding a given animal on a larger amount of carbohydrate than usual does not increase the amylase output; the salivary amylase appears to be a contributing factor to the urine; the differences between various species and also in individuals seem to rest upon factors more fundamental than merely the character of the food, although in the evolution of the form, food may have been a factor.

2. Age and urinary amylase.

In 1873 Korowin (25) reported that the pancreas of infants does not secrete amylopsin until about one month after birth,

[1] The saliva of Mr. C. was much more active than that of either Mr. W. or Mr K. The amylolytic power of his urine was also constantly greater.

and that from that time to the end of the first year the amount gradually increases at which time the full activity of the pancreas is reached. The saliva of infants contains amylase at birth according to this author. This fact perhaps explains why the amylclytic power of the urine is lower than that of adults.

Our experiments were carried out on pups and kittens, neither of which produced any salivary amylase. The experiments were carried out in order to compare the urine of young animals with that of adult animals, and also to determine what part is played by the pancreas in contributing to the urinary amylase. The internal secretion is apparently present in these young animals so that the effects of loss of internal secretion could not be brought in to explain the decreased amylase in the blood and urine.

The experiments on the pups were not so conclusive because in many cases we did not know the exact age, and also because frequently they were in poor condition. The ages of the kittens were better known and the animals were in good condition. Table V is a summary of the results. The six day column is an average of seven different kittens none of which was more than six days old. The sixteen day column is an average of a single litter of four kittens. The six weeks column is an average of three kittens one of which gave urinary values very low, consequently lowering the average readings. We do not consider this of great significance inasmuch as we find variations as great or greater in normal adult cats. The seven weeks column is an average of three kittens of the same litter. These figures conclusively show that at birth and the first few days following birth the pancreas of kittens produces very little amylase, and that soon after there is an increased production which by the sixth week at least, has reached approximately that of the adult cat. There is also a suggestion that the pancreas bears a direct relation to the blood and urinary amylase, and in addition a proof that the pancreas is not the only source in the cat.

3. Source of blood and urinary amylase

Hirata (26), Schirokaner and Wilenko (27), Bradley (28), and Mac Lean (29), have shown that compared with the other organs of the body, the kidneys contain a large amount of amylase, often exceeding that of the liver. This may be a kidney enzyme or it may be derived from the blood and lymph and fixed in some way by the kidney cells. In either case a small amount may gradually find its way into the urine thus making the kidney a direct source. Aside from this, it is quite evident and generally accepted that the urinary amylase is brought to the kidney by the blood and lymph preformed, and eliminated with other organic substances. Thus it appears that the correct answer to the question of the origin of urinary amylase can be reached only by finding the various paths by which amylase gets into the blood.

Our results justify the following conclusions: salivary amylase appears in the urine; the pancreas is a direct source of urinary amylase; and finally, there are other sources than the two just named.

The first conclusion is based upon the fact that those animals which secrete ptyalin, eliminate in the urine more amylase per kilo body weight than those which do not secrete a starch splitting enzyme into the mouth. The second is based upon the observation that the urinary amylase is much less than normal in animals after pancreatectomy, and also in normal young animals before the pancreas becomes fully functional. The last conclusion is based upon the fact that there is not a complete disappearance of urinary amylase in case of absence of both salivary and pancreatic amylase.

Both the salivary glands and the pancreas pour their secretions into the alimentary tract. There are two paths by which the enzymes from these glands may find their way into the blood, either by direct passage from the gland tissue into the blood or by absorbtion from the digestive canal. The experiments in which large amounts of amylase were given to cats per mouth, and also put into the small intestine of the dog, resulting in an

increase of amylase in the urine in both cases, prove that absorption from the digestive tract does occur under certain conditions. The result following ligation of the pancreatic ducts and trauma of the pancreas point to direct passage from the gland tissue into the blood. Some questions may be raised in regard to the latter point, but it is difficult to see how trauma of the pancreas could accelerate absorption from the small intestine, or even how an injured gland could secrete more amylase than a normal one. Furthermore the active hyperemia present in the inflamed gland presents a condition most favorable to local absorption. In case of the amylase derived from sources other than those glands just named there can be no question, for the only path open is that of direct passage into the blood.

So far we have presented proofs that amylase can pass into the blood by two paths, but have not stated which is the more important under normal conditions. We are inclined to the view that the blood receives the enzyme through both channels, but that more is received directly into the blood from the glands than from the digestive canal. A discussion of the reasons for this view opens up the question of the destruction of amylase within the body.

The amount of amylase eliminated in the urine during twenty-four hours must represent only a small fraction of the amount produced in the body during that same time, or even that produced by the pancreas alone. Evidently there is some mechanism adapted to inactivate or destroy amylase. The evidence for and against the existance of antiamylase is conflicting. The works of Kussmaul (30) and of Ascoli and Bofanti (31) appear uncertain, while Preti (32) claims to have demonstrated such a substance as well as its specificity. In our own work we have found no such substance in the blood of normal animals.

In those animals which secrete ptyalin, the enzyme swallowed during meals is probably to a large extent destroyed by the HCl in the stomach. That swallowed into an empty stomach may find its way into the intestine. We know of no evidence for absorption of amylase through the gastric mucosa, but the general behavior of the stomach as an absorptive organ would speak

against it. In the intestine, amylase may disappear in one or a combination of three ways: by way of the feces; by local destruction; and by absorption through the intestinal mucosa. The feces of man and the dog, the only ones tested by us, contain only a small amount of amylase. In diarrhea in dogs with watery stools the amount may be large with a slight decrease in the urine, which agrees with clinical findings in the human. The contents of the whole length of the small intestine during pancreatic activity, whether mixed with food or not are strongly amylolytic. It would be peculiar that the medium in which the enzyme must act would be so destructive. Absorption seems much more probable, in which case we should expect a rise in amylolytic power of the blood and urine after meals. Earlier investigators (33) have reported such an increase. Repeated tests have failed to show such an increase in the blood, but on an average the urine secreted about the third hour after meals is more strongly amylolytic than that secreted during any other hour of the day, although we did not always find this the case. Whatever the facts may be, the increase is in no way commensurate with the amount of enzyme secreted by the pancreas during the digestion of a meal.

It appears then, that the enzyme absorbed from the intestine is almost totally inactivated or destroyed in the intestinal mucosa. This probably, however, is not the only point of destruction for in the case of injection of foreign amylase the disappearance from the blood is a constant phenomenon,—it requiring on an average about eight hours to eliminate 5000 units. Mixture with the lymph and consequently dilution may account for the more rapid decrease during the first hour, the emptying of the first lymph containing an increase in enzyme being indicated by the slight abrupt rise of the blood at about that time (see fig. III). The destruction or elimination of amylase from the blood does not depend in any large measure upon the kidney, for ligation of the renal arteries does not cause a demonstrable rise in the serum (Van de Erve (14)), nor is the elimination of foreign amylase appreciably altered (see fig. III). The process is a relatively slow one for we could find no constant difference

between the amylolytic power of portal and carotid sera. This is in general agreement with the findings of Carlson and Luckhardt (34) who found no difference between portal and hepatic sera.

These facts do not absolutely prove the hypothesis that the urinary amylase represents in a large measure the enzyme finding its way into the blood directly from the tissues, but lends it support. We have proved that amylase does enter the blood directly from the tissues, and that when once in the blood the enzyme is destroyed much more slowly than is the case during its passage through the intestinal mucosa. The amylase feeding experiments indicate that only in case the digestive tract is flooded with the enzyme does enough pass into the blood to bring about a significant increase in the urine.

There are various possible sources of the urinary amylase not accounted for by the salivary glands and pancreas. We have already mentioned the kidney itself as a source. In addition the liver, muscles, spleen and leucocytes contain amylase, which may serve as sources.

It is generally accepted that in the mobilization of glycogen an enzyme which also has the power of splitting starch is involved. During this process a larger amount than normal may pass into the blood. We have repeatedly observed that in animals under ether anesthesia the appearance of sugar in the urine is accompanied by an increase in amylase. Also, frequently we have found in animals just picked up from the street, a relatively high amylase output during the first few days in the cage, followed by a rapid fall to the normal laboratory level, which suggests that the rapid metabolism incident to greater bodily activity bears some causal relation to the amylase output. A series of six day tests on three men failed to show any parallelism between the nitrogen metabolism and the amylase output in the urine.

The leucocytes as a source of amylase must not be overlooked. It is known that they contain the enzyme. Even under normal conditions their destruction is relatively rapid. The increase of amylase in the blood and urine of animals dying of acute infections may be due to the greater destruction of the leucocytes accompanying the leucocytosis.

4. Significance of variations in urinary amylase

Throughout this paper we have used the term amylase always in the singular, not because we believe there is only one starch splitting substance in the body, but because we have no means of definite identification. By the term amylase we mean a substance which carries the hydrolysis of starch to the achroodextrin stage at least. Consequently, by variations we mean quantitative rather than qualitative variations.

The evidence already presented and discussed indicates that fluctuations in the amylolytic power of the urine may be due to increased or decreased production of amylase within the body, to an obstruction to the normal outflow from the pancreas or salivary glands, to injury of the pancreas, to decreased absorption from the intestinal lumen, to the liberation of glycogenolytic enzyme, and possibly to leucocytosis.

The results obtained from animals dying after a period of depression are difficult to interpret because of so many abnormal factors. We have already suggested the possible role of leucocytosis. In addition to this, a certain amount of autolysis takes place resulting in the liberation of intracellular enzymes. These animals as a rule take very little food or water, but we have proved that in water starvation, with the elimination of a highly concentrated urine, the amylase is decreased. Neither is it probable that there is an increased production of amylase. The resistance of these animals is very low and possible their power to destroy amylase is decreased in the same proportion. Finally, we must not overlook the possibility of a terminal infection being the immediate cause of death in these depressed animals.

We have assumed that urinary amylase is identical with that in the blood and lymph. So far in our discussion we have confined ourselves largely to the questions of the origin, paths of absorption, and destruction of amylase. We have proved that the kidney plays a small part in the process of elimination of enzyme from the blood. Shifting the viewpoint, it becomes evident that the amylase in the urine must bear an important relation with the physiological activity of the kidney.

The results indicate that the kidneys take an active part in the elimination of amylase. If this were not the case then it is difficult to see how the concentration in the urine could under any condition exceed that of the blood. We found this condition in the dogs with the pancreatic ducts ligated (fig. I), and also in a dog dying after depression (table VII). We also have observed an increase of amylase in the urine in caffeine diuresis. The results obtained from animals with tartarte nephritis, although not yet completed, indicate a parallelism between the rate of urinary secretion and amylolytic power.

A change in the permeability of the kidney, such as is obtained after injection of phloridzin, results in an increase of amylase in the urine (fig. II).

In conclusion, it is evident that the variations in the amount of amylase in the urine, independent of the amount in the blood, may depend upon the permeability of the kidney, the condition of the renal epithelium, and upon the normal physiological variation in renal activity. It is possible that amylase may act as a stimulus resulting in its own elimination just as is urea or dextrose.

It thus appears that quantitative estimations of urinary amylase alone would have but little significance as a diagnostic point, but that a long series of tests coupled with an accurate history may serve as a useful supplement in the diagnosis of renal lesions, pancreatic lesions, and obstructive lesions.

5. Role of blood and urinary amylase.

Abderhalden and Brahm (18) have classed this enzyme as a protective ferment, not finding it normally in the blood, but appearing after the subcutaneous injection of starch, the curve of appearance and increase resembling that of a typical immunity reaction.

We have found blood and urinary amylase present in all the animals tested. We also observed an increase in the urine of animals after intravenous injection of starch, but the increase reached the maximum height about the second day after the

injection, showing the increase not to have been due to a typical immunity reaction.

We have no data which would either prove or disprove the contention of Abderhalden and Brahm (18) that parenteral administration of starch stimulates the formation of amylase which may act as a protective enzyme. We are at a loss, however, to understand how these investigators could find amylase normally absent from the blood, unless their method is accountable. The optical method depends upon variations in rotation. We have found that frequently no reducing sugar is demonstrable after the incubation of starch with serum, consequently the changes in rotation may be small. The work here reported confirms the contention of Bial (35), Hamburger (36), Borchardt (37), Ascoli and Bofanti (31), Carlson and Luckhardt (34) and many others.

The amylase in the blood and urine appears to play no important role, but having found its way into a medium normally containing no substrat, is destined to destruction and elimination. Incidentally it may be a contributing factor in maintaining colloid equilibrium in the blood, which may account for the relative constancy of concentration.

SUMMARY AND CONCLUSIONS

1. There are variations in the amylolytic power of urines from different species, and urines from individuals of the same species, not accounted for by differences in diet and feeding habits.

2. The amylase output in the urine per kilo weight is greater in those animals which secrete both salivary and pancreatic amylase than in those secreting only the latter.

3. In young animals the increase in urinary amylase runs parallel with the increase of pancreatic activity.

4. The digestive glands pouring amylase into the alimentary tract are not the only source of enyzme finding its way into the urine.

5. Swallowed ptyalin is probably nearly all destroyed by the HCl in the stomach.

6. The amylase of the intestinal canal is probably absorbed and either destroyed or inactivated during its passage through the intestinal mucosa.

7. Lesions causing obstruction to the natural outflow of the pancreatic juice lead to an increase of amylase in the urine.

8. Traumatic injury to the pancreas results in an increased amyloloytic power of the urine.

9. Pancreatectomy results in a decrease in urinary amylase.

10. The kidneys play a minor role in the destruction and elimination of amylase.

11. The amount of amylase in the urine depends upon the amount finding its way into the blood, and upon the functional capacity of the kidney.

12. The increase of amylase in the urine and sometimes in the blood in cachexia is of significance and must be taken into consideration when applying the amylase test as a diagnostic measure.

13. The conclusion of Abderhalden and Brahm, that amylase appears in the blood only after parenteral administration of starch has not been confirmed.

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THE EFFECT OF FILTRATION THROUGH A BERKEFELD FILTER UPON THE COAGULABILITY OF OXALATED PLASMA

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In a paper by Cramer and Pringle[1] it is stated that centifugalized oxalated plasma, after filtration through a Berkefeld filter, will not clot upon the addition of calcium chloride. The authors interpret this result to mean that oxalated plasma, after ordinary centrifugalization, still contains some blood platelets. Addition of calcium chloride to unfiltered oxalated plasma brings about prompt clotting owing to the fact that the platelets are caused thereby to undergo disintegration with liberation of thrombokinase. After passing through a Berkefeld filter the plasma is deprived completely of the platelets and addition of calcium salts therefore is without effect in inducing coagulation. It follows further from this point of view that normal circulating blood contains no thrombokinase (or thromboplastin), and that therefore the blood platelets are of essential importance in initiating the process of coagulation. Thrombokinase is "liberated only by the breaking up of the platelets." The authors admit that filtration through a Berkefeld filter does not always deprive the oxalated plasma of its property of clotting upon the addition of calcium salts, but they claim that in these exceptional cases the clotting is at least delayed for many hours, whereas under similar conditions the unfiltered plasma clots within a few minutes. In one case which they mention specifically the blood taken from an animal three hours after a meal was but little affected by the filtration. The filtered plasma, on addition of calcium salts, clotted only five minutes after the unfiltered plasma.

[1] Quarterly Journal of Experimental Physiology, 1913, vol. vi, p. 1.

In view of the importance of these results in their bearing upon the composition and coagulability of normal circulating plasma I was requested by Professor Howell to repeat the experiments. It may be stated at once that my experiments have shown that Cramer and Pringle's main conclusion, namely, that filtered oxalated plasma "does not clot on the addition of soluble calcium salts" is correct only when the first portions that come through the Berkefeld-filter are used. The very first filtrate indeed not only does not clot upon the addition of calcium salts, but may fail to clot upon adding an active solution of thrombin, thus indicating that fibrinogen is absent. The intermediate portions of the filtrate may either not clot at all upon the addition of calcium salts or may show a delayed coagulation of variable amount, while the last portions of the filtrate clot quite promptly when calcium chloride is added. The results of all of the experiments were concordant and may be presented very briefly.

METHOD

In the first experiments, made upon cats, great care was taken to restrain as far as possible the breaking down of the platelets in the oxalated blood. The animals were bled from the carotid through a vaselined glass tube, and the blood was received into the oxalate solution placed in a paraffined centrifugal tube. The oxalate solution consisted of a 1 per cent solution of sodium oxalate made up in a 0.9 per cent solution of sodium chloride—the amount used being sufficient to give a final mixture in the oxalated blood of 0.1 per cent sodium oxalate. The oxalated blood was centrifugalized for thirty minutes at a speed of 3000 revolutions per minute, and the upper portions of the plasma were then sucked off through a vaselined aspiration tube into a vaselined test tube. This plasma was then sucked through a Berkefeld filter. The glass cylinder of the Berkefeld filter was also coated with vaseline before use. About 30 to 40 cc. of the plasma were aspirated through the filter and the filtrate was divided into three or four parts of approximately 10 cc. each and labelled, *a*, *b*, *c*, *d*; *a* being the first portion to come through and *d* the last. The Berkefeld

filter was in some cases used dry, after sterilization in an autoclave; in other cases it was first moistened with a 0.1 per cent solution of sodium oxalate in 0.9 per cent solution of sodium chloride. It was found later that the elaborate precautions used to vaseline or paraffine all surfaces with which the blood came into contact before filtration were not essential. Blood caught through a vaselined tube into oxalate solution contained in a clean centrifugal tube and put through the above procedure gave identical results.

After each experiment the Berkefeld filters were cleaned by sucking through them a large volume of dilute solution of sodium hydroxide and subsequently distilled water. The filters were then sterilized in the autoclave.

The filtrate was examined as to its coagulability by testing it with optimum amounts of a 0.5 per cent solution of calcium chloride, with and without previous dilution of the filtrate with an equal volume of water; by the addition of fresh and boiled tissue-extracts (extract of cortex of the brain in 0.9 per cent solution of sodium chloride), and by the addition of a solution of the phosphatid (kephalin) which Howell has shown is the essential constituent in tissue extracts, so far as their effect in promoting coagulation is concerned.

It will be seen from this table that the first portion of the oxalated plasma that passes through the filter (Exps. II, III, V, VI) shows no clotting upon the addition of calcium salts, nor upon the addition of calcium salts plus thromboplastic substance as furnished by brain extract or solutions of kephalin. A later filtrate may show no clotting upon the addition of calcium salts alone, but clots promptly when calcium plus thromboplastic substance is added, while a later filtrate still may clot readily upon the addition of calcium salts alone as in the case of the unfiltered plasm. Moreover, in the case of the first filtrate addition of thrombin (Exp. II, portion a) may not cause clotting, showing that no fibrinogen is present. The filtrate in this instance gave only a very minute precipitate upon the addition of excess of alcohol, indicating that it contained very little protein of any kind.

It may be said that whenever the filtrate contained sufficient fibrinogen to give a clot addition of calcium alone or of calcium

EXPERIMENT	PLASMA	CALCIUM CHLORIDE 0.5 PER CENT	WATER	KEPHALIN SOLUTION	BRAIN EXTRACT	CLOTTING TIME
	cc.	cc.	cc.	cc.	cc.	
I	Unfiltered 0.3	0.2				4 minutes
	Filtered					
	Portion a. 0.3	0.2				No clot in 4 hrs. 30 min.
	Portion a. 0.3	0.2	0.3			No clot in 4 hrs. 30 min.
	Portion a. 0.3	0.2		0.3		14 minutes
	Portion b. 0.3	0.2				1 hour
	Portion b. 0.3	0.2	0.3			20 minutes
	Portion b. 0.3	0.2		0.3		3 minutes
II	Unfiltered 0.3	0.2				4 minutes
	Filtered					
	Portion a. 0.3	0.2				No clot in 24 hours
	Portion a. 0.3	0.2	0.3			No clot in 24 hours
	Portion a. 0.3	0.2		0.3		No clot in 24 hours
	Portion b. 0.3	0.2				No clot in 24 hours
	Portion b. 0.3	0.2	0.3			No clot in 24 hours
	Portion b. 0.3	0.2		0.3		30 minutes
	Portion b. 0.3	0.2			0.3	4 minutes
	Portion c. 0.3	0.2				15 to 30 minutes
	Portion c. 0.3	0.2	0.3			15 to 30 minutes
	Portion c. 0.3	0.2		0.3		4 to 6 minutes
III	Unfiltered 0.3	0.2				5 minutes
	Filtered					
	Portion a. 0.3	0.2				No clot in 24 hours
	Portion a. 0.3	0.2	0.3			No clot in 24 hours
	Portion a. 0.3	0.2		0.3		No clot in 24 hours
	Portion a. 0.3	0.2			0.3	No clot in 24 hours
	Portion b. 0.3	0.2				No clot in 24 hours
	Portion b. 0.3	0.2	0.3			No clot in 24 hours
	Portion b. 0.3	0.2		0.3		Partial clot in 10 minutes
	Portion b. 0.3	0.2			0.3	3 minutes
	Portion c. 0.3	0.2				1 hour
	Portion c. 0.3	0.2	0.3			1 hour
	Portion c. 0.3	0.2		0.3		6 minutes
	Portion c. 0.3	0.2			0.3 (boiled)	1½ minutes

EXPERIMENT	PLASMA	CALCIUM CHLORIDE 0.5 PER CENT	WATER	KEPHALIN SOLUTION	BRAIN EXTRACT	CLOTTING TIME
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	
IV	Unfiltered 0.3	0.2				5 minutes
	Filtered					
	Portion a. 0.3	0.2				No clot in 20 hours
	Portion a. 0.3	0.2	0.3			No clot in 20 hours
	Portion a. 0.3	0.2		0.3		20 minutes
	Portion a. 0.3	0.2			0.3	4 minutes
	Portion b. 0.3	0.2				40 minutes
	Portion b. 0.3	0.2	0.3			30 minutes
	Portion b. 0.3	0.2		0.3		9 minutes
	Portion b. 0.3	0.2			0.3	2 minutes
	Portion c. 0.3	0.2				10 minutes
	Portion c. 0.3	0.2	0.3			10 minutes
	Portion c. 0.3	0.2		0.3		4 minutes
	Portion c. 0.3	0.2			0.3	1 minute
V (In this experiment only the cannula was vaselined.)	Unfiltered 0.3	0.2				8 minutes
	Filtered					
	Portion a. 0.3	0.2				No clot in 3½ hours
	Portion a. 0.3	0.2	0.3			No clot in 3½ hours
	Portion a. 0.3	0.2		0.3		No clot in 3½ hours
	Portion a. 0.3	0.2			0.3 (boiled)	No clot in 3½ hours
	Portion b. 0.3	0.2				No clot in 3½ hours
	Portion b. 0.3	0.2	0.3			No clot in 3½ hours
	Portion b. 0.3	0.2		0.3		12 minutes
	Portion b. 0.3	0.2			0.3 (boiled)	5 minutes
	Portion c. 0.3	0.2				2 hours
	Portion c. 0.3	0.2	0.3			1 hour
	Portion c. 0.3	0.2		0.3		7 minutes
	Portion c. 0.3	0.2			0.3	2½ minutes
	Portion d. 0.3	0.2				30 minutes
	Portion d. 0.3	0.2	0.3			24 minutes
	Portion d. 0.3	0.2		0.3		8 minutes
	Portion d. 0.3	0.2			0.3 (boiled)	2½ minutes

EXPERIMENT		PLASMA	CALCIUM CHLORIDE 0.5 PER CENT	WATER	KEPHALIN SOLUTION	BRAIN EXTRACT	CLOTTING TIME
		cc.	cc.	cc.	cc.	cc.	
In this experiment lymph was used. The lymph was obtained from the thoracic duct of a dog through a vaselined cannula and was oxalated and centrifugalized	VI	Unfiltered					
		0.3	0.2				80 minutes
		0.3	0.2	0.3			50 minutes
		0.3	0.2		0.3		8 minutes
		0.3	0.2			0.3 (boiled)	5 minutes
		Filtered					
		Portion a. 0.3	0.2				No clot in 20 hours
		Portion a. 0.3	0.2	0.3			No clot in 20 hours
		Portion a. 0.3	0.2		0.3		No clot in 20 hours
		Portion a. 0.3	0.2			0.3	No clot in 20 hours
		Portion b. 0.3	0.2				No clot in 20 hours
		Portion a. 0.3	0.2	0.3			No clot in 20 hours
		Portion b. 0.3	0.2		0.3		10 minutes
		Portion b. 0.3	0.2			0.3 (boiled)	10 minutes

plus thromboplastic substance (tissue-extract, kephalin) gave a clot.

In the light of these results it is evident that the interpretation placed upon their results by Cramer and Pringle, namely, that the absolute or relative incoagulability of the filtrate is due entirely to the removal of suspended platelets, is not admissible. The filter holds back, for a time, not only suspended elements, but the various proteins in colloidal solution in the plasma. In several cases that portion of the filtrate, which would not clot with calcium salts alone but gave a clot upon the addition of calcium salts and thromboplastic substance, was examined for prothrombin by Professor Howell, making use of a method to be described in a forthcoming paper. The test showed the presence of prothrombin as would be expected. So far as these experiments go they indicate that of the various proteins concerned with the process of coagulation the fibrinogin, antithrombin and prothrombin penetrate the filter more readily than the thromboplastic substance (thrombokinase of Morawitz).

Instead of assuming as is done by Cramer and Pringle that

thrombokinase (thromboplastic material) is not present in solution in the circulating plasma, but is contained in, and, so far as the blood is concerned, is furnished only by the platelets, the reverse is more probable, namely, that this material is constantly present in solution in the plasma, but owing to its condition of dispersion it is strained off more completely or for a longer time by the Berkefeld filter than the fibrinogen or the prothrombin. It will be noted, for instance, that the plasma of the oxalated lymph, which contains no platelets, behaves as regards the coagulation of the successive filtrates in the same way as the oxalated blood-plasma. The platelets do contain an abundance of thromboplastic material, and in the act of disintegration furnish it to the plasma. This reaction, in fact, is important or even essential to the prompt clotting of blood. But the evidence makes it probable that some of this material is present normally in the circulating blood. At any rate the experiment reported by Cramer and Pringle furnishes no conclusive proof of the necessity of an organic kinase, in addition to calcium, in the activation of prothrombin. The behavior of those portions of the filtrate that do not clot with calcium alone, but coagulate readily with calcium salts and brain extracts or kephalin solution, is entirely explained in terms of Howell's theory of coagulation. Such filtrates contain an excess of antithrombin and the kephalin or brain extracts permit clotting by neutralizing the effect of the antithrombin, and thus allowing the calcium to activate the prothrombin to thrombin.

IS THE CONTRACTION OF SMOOTH MUSCLE ACCOMPANIED BY HEAT PRODUCTION? FIRST COMMUNICATION

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INTRODUCTION

The great rôle smooth muscle plays in blood vessels, in the digestive tract, and in the urino-genital system has impressed physician and physiologist alike. And yet in spite of the penetrating studies of such authors as Bethe[1] (1903), Grützner[2] (1904) and von Uexküll[3] (1905–1908), an intimate knowledge of this tissue is wanting at many points.

The most important side of the physiology of a muscle tissue (and therefore of a muscular organ) is, beyond a doubt, that part which may be referred to as its energetics.

No theoretical considerations can be of permanent value unless based upon actual determinations of energy exchange. For cross-striated muscle such determinations are now comparatively numerous, although much is still to be done. For smooth muscle, however, measurements of energy exchanges have been very few and it was in the hope of being able to add to the data of this kind that the present work was undertaken.

[1] Bethe, A.: Allgemeine Anatomie u. Physiologie des Nervensystems. Leipzig, 1903, p. 363.

[2] Grützner, P.: Ergebnisse der Physiologie. Wiesbaden, 1904, iii, part 2, p. 12.

[3] von Uexküll, J.: Zeitschrift für Biologie, 1899, xxxix, p. 77; "Umwelt u. Innenwelt der Tiere," 1909, p. 91.

THE LITERATURE

No attempt will be made to review in any exhaustive way the literature bearing upon the subject matter of the present paper. That fortunately has been done almost to date by other writers.[4] On the contrary only the work of a few authors will be mentioned and that only in part, in order to point the way in the new field and to lay emphasis upon the meaning and interpretation of the results here recorded.

For smooth muscle, it seems that the first observations on energy exchanges are those of Cohnheim[5] (1908) who measured the CO_2 output of surviving cat gut. This author's work led him to the conclusion that smooth muscle expends only about one-tenth as much energy as the same amount of cross-striated muscle for corresponding amounts of work. Kehrer[6] (1909) measured the CO_2 output of cat's uterus and found the normally contracted organ gave off 10 to 15 mgm. per hour; that of the extended organ in the pregnant animal gave off 20 to 30 mgm. per hour; and that directly after parturition the output diminished practically to the zero point.

More recently Parnas (1910) and Bethe (1911) have attempted to give sharper definition to our knowledge of the gaseous metabolism of smooth muscle. Parnas[7] determined the respiratory quotient of intact bivalve molluscs while the animals were stimulated to keep their valves closed (1) against a weight of 3000 grams, (2) against a weight of 300 grams. No appreciable difference in the respiratory quotient was observed in the two cases. Indeed this author found that the total gaseous metabolism of molluscs with their valves held shut against heavy loads for long periods of time was so small that that portion belonging to the adductor muscle must be about $\frac{1}{50,000}$ part of the metabolism of cross-striated muscle of similar dimensions and under the same conditions.

[4] For bibliographies on smooth muscle see, besides Grützner, P., loc. cit., also Parnas and Noyons u. von Uexküll, both cited below.

[5] Cohnheim, O.: Zeitschrift für physiologische Chemie, 1908, liv, p. 461.

[6] Kehrer, E.: Archiv für Gynäkologie, 1909, lxxxix, p. 574.

[7] Parnas, J.: Archiv für die gesammte Physiologie, 1910, cxxxiv, p. 441.

Bethe[5] observed that Unio starving for 24 days and with adductor muscles contracted against a heavy load lost no more in body weight (either wet or dry substance) than those which starve for the same length of time with the adductors free and unweighted. If a man's cross-striated muscle of same size had contracted against a similar load for the same time an amount of sugar would have been consumed equal to 8.8 times the weight of the bodies of the molluscs. In other experiments Bethe found that the O_2 consumption of Unio with the adductors contracted against a slight weight was the same as when contracted against a heavy load.

Measurements of the gaseous metabolism of specimens of Aplysia, (1) intact and at rest, (2) with the large mass of smooth muscle of the mantle thrown into permanent "tetanus" (by removal of the oesophageal ring and ganglia) showed no difference in the O_2 consumption or the CO_2 output.

From these results Bethe makes some interesting calculations. If the arteries in the human body maintain their normal tension on the basis of energy exchanges such as is involved in the maintenance of tension by cross-striated muscles they must require from one-sixth to one-fourth (I can make it only one-tenth from the data) of the daily energy exchange of the whole body at rest. His observations upon the fatiguability of smooth muscle in Unio, and the deductions that he makes therefrom, are not less interesting. For the data and his methods of calculation the reader must consult the original paper. It must suffice here to mention only his conclusions, namely, that a frog's gastrocnemius fatigues 1,000,000 times more quickly than a mussel's adductor muscles, and 30,000,000 times more easily than the musculature of a rabbit's blood vessel; a man's biceps fatigues from 8700 to 51,000 times more quickly than a Unio's adductors and 1,400,000 times more quickly than a rabbit's blood vessel.

The experimental evidence thus seems to point unanimously to the conclusion that smooth muscle maintains its tension at

[5] Bethe, A.: Archiv für die gesammte Physiologie, 1911, cxlii, p. 291.

the cost of much less oxidation of materials than does cross-striated muscle.

Since the metabolism of contracting smooth muscle seems to be comparatively low, is the accompanying heat production likewise correspondingly low? Or is any heat at all produced by smooth muscle when it contracts?

The experiments about to be described were carried out specifically to answer these questions.

EXPERIMENTAL

Apparatus and method. The choice of apparatus and general procedure was based chiefly upon the experience and recommendations of Bürker[9] (1900–1908).

The thermoelectric method of measuring small heat changes was adopted. The style of thermopile chosen was the differential or "Gittersäule" of Bürker with some modification in the form and material of the frame upon which the wires were wrapped.

Several sizes were made, one of 32 junctions, one of 48, and two of 60 junctions. Care was taken to use the minimum amount of silver in soldering the junctions. The metals used were iron and constantan wires of 0.0873, or 0.126 mm. diameter. The resistances of the thermopiles vary from 8 to 25 ohms.

A Paschen[10] galvanometer was employed whose coils in series have a resistance of 12 ohms; in parallel, a resistance of 0.75 ohms.

A detailed description of the construction of the thermopiles, special copper keys, and other accessory apparatus, which proved to be best fitted for the work, must be left for some future paper.

It was thought desirable to know the absolute value of the thermoelectric effects, as recorded by the deflections of the galvanometer, in terms of actual heat units. The method adopted for this was in part that of A. V. Hill[11] (1913), which consists of passing a known quantity of electricity through the experimental muscle after killing it and while it is still in position on the ther-

[9] Bürker, K.: Tigerstedt's Handbuch der physiologischen Methodik, 1908, ii, part 3, pp. 1–86.

[10] Paschen, F.: Zeitschrift für Instrumentkunde, 1893, p. 13.

[11] Hill, A. V.: Journ. of Physiol., 1913, xlvi, p. 435.

mopile. The heat thus produced in the muscle causes the galvanometer mirror to deflect a certain number of scale divisions. The resistance of the muscle being measured in ohms, and the strength of current used and the time of its passing, being known, one can calculate the heat produced in calories by the usual formula.

The difficulties are numerous and have been reviewed by Hill in the article above referred to.

In the present series of experiments it has been found quite satisfactory to use the tetanizing induced current from an inductorium such as one usually finds in physiological laboratories. The same current can be used for stimulation of the muscle, direct or indirect, and for heating it when desired; also for determining the resistance of the muscle.

The difficulty in using such a current is to know its exact strength, and (when passing it), the exact amount of electricity degraded in the muscle, without which the current would be quite useless.

This difficulty was surmounted by using a Rowland electro-dynamometer, a model of the instrument which was especially designed by the late Professor Rowland[12] for physiological work in this laboratory. The instrument has been briefly described elsewhere[13] as an instrument for physiological research and will not be described further here, excepting in so far as it concerns the experiments in hand.

With the electro-dynamometer in circuit, and with the proper shunts and resistances one can read off practically directly the amperage of any ordinary sort of alternating or direct current. The instrument may be used to determine the resistance of the muscle under investigation at any time by simply balancing known resistances against that of the muscle with the electro-dynamometer in circuit, until the readings are equal.

As was stated the tetanizing current from an induction coil was used. This same current was used for stimulating the muscle

[12] Rowland, H. A.: American Journal of Science, 1897, iv, p. 429.
[13] Greene, C. W.: This Journal, 1898, i, p. 106.

and for electrical heating of the muscle, as well as for the determination of its resistance.

Since the amperage of the current could thus be determined it was only necessary to know that part of the resistance in the circuit represented by the muscle, and the duration of the heating in seconds. The amount of heat in calories produced by the current passing through the muscle may then be calculated from the formula.[14]

$$h = i^2 R_m t \times \frac{1}{4.19}$$

in which h represents the heat in calories,

i, the amperage of the current,

R_m, the resistance of the muscle in ohms, and

t, the time the current passes in seconds.

Both i and R_m are measured by means of the electro-dynamometer.

It may be added that the virtual electromotive force of the tetanizing current, with the secondary coil of the inductorium at various distances from the primary coil, may also be determined by the electro-dynamometer. In other words one may calibrate the inductorium in terms of virtual electromotive force and virtual resistance. The virtual resistance is gotten out of certain constants obtained during the calibration process, from which one may make the calculation. The details of this method of using the electro-dynamometer in physiological work, however, must be left for a later communication.

The chief disadvantage in the use of the electro-dynamometer is that it requires 10 to 15 seconds to make a reading, and therefore this instrument cannot be read off during the passage of a current of shorter duration. But since it gives the value of the current's

[14] The factor, more exactly, 0.2385, or $\frac{1}{4.1914}$ in this equation is the reciprocal of "Joule's Equivalent" or the "Mechanical Equivalent of Heat" as determined by Rowland, H. A.: Proceedings of the American Academy, 1880, xv, p. 75. The more recent determinations by Barnes (Phil. Trans. Roy. Soc., A, 1902, cleix, p. 149, and Proc. Roy. Soc., A, 1909, lxxxii, p. 390) give this factor the value of 4.1873 which for the present work is practically the same number as Rowland's.

strength which remains independent (within certain limits) of the duration of its passage, the reading can be made just before or after, or at intervals, during an experiment.

But if the values E_v and R_v of the secondary coil have been previously determined, the stimulating current may be thrown into the muscle directly, i.e., with the electro-dynamometer out of circuit, for any duration of time desired. The formula used in this case for the calculation of the heat produced by the current is

$$h = \left(\frac{E_v}{R_v}\right)^2 R_m \times 0.239\, t,$$

where R_m only is read off, as before, at the end of the experiment, on the electro-dynamometer.

The induction coil used in most of the experiments was a beautiful type of the Gaiffe inductorium with secondary coil No. 14. The sliding carriage for the secondary coil in this instrument is already carefully calibrated in Kronecker units which makes the calibration in absolute values of E_v much simpler.

In the present investigation it was desirable to allow the muscle to develop tension but not to change its length. The records therefore were taken for the most part with an isometric writing lever attached.

This lever was connected by a loose joint to a short piece of piano wire stretched in the frame of a small "leaf saw." The point of attachment of the lever to the piano wire was 1 mm. distant from the fulcrum and 300 mm. distant from the writing point. The lever was carefully calibrated from time to time, so that the excursions of the writing point caused by various possible tensions exerted by the muscle could be easily converted into terms of grams.

The lever was so adjusted as to allow the muscle to shorten only about 0.06 mm. when exerting its greater tensions. In which case the writing point of the lever passed through an excursion of 20 mm.

As to the moist-chamber, precautions about thermostatic and hydrostatic conditions; about possible short-circuiting of current,

thermo-electric, inductive and capacity effects escaping detection—all these were controlled with painstaking care.

As sources of error they have been dwelt upon by other authors and need be given no space here.

Material The muscles chosen for the experiments was the bladder of small turtles, supplied by the market, so called "box turtles," "snappers" and terrapin.

For purposes of control and as a measure of the efficiency of the instruments the gastrocnemius and sartorius muscles of the frog were also used.

The bladder of a turtle of 6 to 8 inches in body length is beautifully adapted for comparison with a pair of gastrocnemius or sartorius frog muscles, because when empty the organ shows a perfect bilobed division of its musculature. The two lobes are united at the neck of the bladder. When the organ is excised it may be hung astride a flat (Gittersäule) thermopile with one lobe covering the "heat junctions" on each side (as does a pair of sartorius of gastrocnemius muscles), with the "cold junctions" left free and far enough away in the space of the moist chamber.

The neck portion of the bladder affords a part well suited for purposes of *indirect* stimulation, and at the same time a point for fixed attachment for the preparation. The tips of the lobes are connected to the muscle lever by means of silk ligatures.

The change of length of the fibers in turtle's bladder is something enormous. I have seen the lobes, extended with urine and semen to a length of about 60 mm., contract down upon emptying to a length of 15 or 20 mm. This great change in length I have not yet reproduced on the surviving organ; with a light isotonic lever attached to the muscle, however, one may obtain an actual shortening of nearly 50 per cent of the original length.

The muscles were made to contract by tetanizing with various strengths of current, principally with a strength that may be termed just adequate to provoke maximal contraction.

The stimulus was applied in two ways (1) indirect, and (2) direct. In measurement of heat production of muscle these methods are widely different in certain respects, but ought, if

one could control all the factors involved, give like results. At least the results ought to be near enough for the one set to give some evidence as to the accuracy of the other.

In using the indirect method one has the advantage of the absence of heat of electrical origin in the total heat production registered by the galvanometer. With the current strength small enough and the area of tissue between the stimulating electrodes far enough away from the thermopile, one can be sure, indeed one can demonstrate on the dead muscle, that no heat of electrical origin is registered by the galvanometer. Whatever heat is thus registered must be of muscular origin.

On the other hand by the method of direct stimulation one has the advantage of having a means of generating independently of the muscle heat, enough heat to overcome the inertia of the heat-measuring apparatus, however small this inertia may already be. With the swinging system once in motion a small amount of additional heat say of muscular origin may thus make an increased and measureable deflection of the galvanometer.

To observe the deflection of the galvanometer caused by the amount of heat due to the stimulating current only, one passes the same current after the muscle is dead.

The difference in the deflections would be an index of the amount of heat exchange due solely to the muscle's activity.

The deflection of the galvanometer obtained by stimulating the live muscle *directly* may be represented by h; the deflection when the muscle is dead may be represented by h_e; $h - h_e$ then would equal h_m, that is, the heat of muscular origin. If $h - h_e = 0$, then h_m is zero; if $h - h_e > 0$, then h_m is positive; and again (what may not be impossible) if $h - h_e < 0$, h_m must be negative and the muscle instead of evolving heat absorbs heat as a result of its intrinsic activities.

In the case of stimulating muscle *indirectly* with just adequate stimuli, the total deflection of the galvanometer h would be equal to h_m whatever that be. That is $h = h_m$ and h_m either $= 0$ or is $<$ or > 0.

In applying the two methods with cross-striated muscle on the thermopile one always finds in the case of indirect stimulation that $h = h_m$ and that (given the muscle is irritable and the stimulus adequate) $h_m > 0$, that is, the muscle *evolves* heat during its development of

tension. In the case of direct stimulation of cross-striated muscle $h - h_e = + h_m$. Indeed as will be shown h_m may be equal to $10000h_e$ or more.

Of course if h_e is chosen very large and h_m turns out to be relatively very small, the latter may be masked in the readings of direct stimulation, and one may not be able to determine it at all. Again in the case of *indirect* stimulation if h_e be very large a portion of it by radiation or conduction may reach the thermopile junctions unevenly and thus viciate the values of the readings. For h would no longer be equal to h_m but rather equal to $h_m + \partial h_e$.

The above discussion may seem unnecessary and tedious but the character of the results obtained from this study of smooth muscle are striking enough it was thought to warrant its insertion.

THE RESULTS

Of indirect stimulation. When stimulating the turtle's bladder indirectly, that is through its neck, the results were much more uniform than when stimulated directly. *By the indirect method no positive heat production could be demonstrated.*

A summary of results is tabulated below.

TABLE I

Turtle's bladder muscles

DATE OF PROTOCOL, 1914	WEIGHT OF MUSCLE IN GRAMS	TENSION IN GRAMS EXERTED BY MUSCLE WHEN CONTRACTING	VALUE OF 1 CM. DEFL. OF GALV. IN DEG. TEMP. $\times 10^6$	ACTUAL DEFL. OF GALV. IN S. D. OF 1 CM. EACH
January 17	*ca.* 1.00	25.0	2.0	0
		42.0	2.0	0
		42.0	2.0	0
		100.0	2.0	0
February 16	1.28	12.0	1.6	0
		12.0	1.6	0
May 21	0.58	22.5	3.4	0
June 1	*ca.* 1.00	7.0	2.6	0
		12.0	2.6	0
		18.0	2.6	0
July 10	2.43	20.0	2.1	0
July 14	1.18	+	2.5	0
		+	2.5	0

The value of the galvanometer deflection in terms of degrees of temperature is indicated in the fourth column. As is seen in the fifth column, no deflection of the galvanometer accompanied the development of tension in the muscle even when that tension was as great as 100 grams.

From these results one may not say that no heat is evolved by smooth muscle when exerting tension, but rather that, if heat be evolved 1 gram of muscle substance when exerting 100 grams tension, or less, probably does not yield enough heat to raise its temperature 0.0002°C.

TABLE II

Turtle's bladder muscle when dead

Heated by known amounts of electricity

DATE OF PROTOCOL, 1914	WEIGHT OF MUSCLES IN GRAMS	HEAT PRODUCED IN MUSCLE BY ELECTRIC CURRENT IN CAL. $\times 10^6$	DEFL. OF GALV. IN S. D. (1 CM.) (SPOT PROJECTED 4½ M.)	INCREASE OF TEMP. OF MUSCLE IN DEG. C. $\times 10^6$	VALUE OF 1 S. D. DEFL. IN DEG. C. $\times 10^6$
January 17	*ca.* 1.0			[15]	
		134.0	46	134.0	2.90
		139.0	96	139.0	1.40
		213.0	140	213.0	1.50
		231.0	130	231.0	1.70
March 9	0.82	19.0	0	23.2	0.00
		72.0	24	88.5	3.56
		118.3	80	140.0	1.75
		177.8	200	216.0	1.08
		247.0	300	301.0	1.00

The column of zeros under the heading galvanometer deflection may lead one to suspect that the apparatus was not in working order. A glance at table II showing it at work when the muscles are actually heated (by the electric current) in known amounts will dispel that suspicion.

Dead muscles produce no tensions when an electric current passes through them, but the energy of the current is degraded into heat. In Table II samples of determination of the sensitivity of the apparatus by use of the experimental muscle, after killing it, are given. The

[15] The specific heat of the muscle is taken as unity for this calculation.

currents sent through the dead muscle were of the same strengths used to stimulate the live muscles. In no case did the heating cause a rise of temperature to exceed 0.03°C. and yet the galvanometer deflection was as much as 300 s.d., giving an extreme sensitivity of 0.0001°C. per scale division deflection.

Cross-striated muscle put in the same apparatus when stimulated indirectly gives us opportunity to see the apparatus responding to heat of purely muscular origin.

Excerpts of the protocols using cross-striated muscle are shown here to enable one (1) to judge of the efficiency of the instrumentarium and (2) to give something with which one may compare the results obtained with the smooth muscle.

TABLE III

Surviving cross-striated muscle

Stimulated through nerve. Isometric contraction

DATE OF PROTOCOL, 1914	WEIGHT OF MUSCLES IN GRAMS	H. P. DUE TO ELECTRIC CURRENT IN CAL. $\times 10^4$	DEFLECTION OF GALVANOMETER IN S. D.	VALUE [16] OF SAME IN DEG. C. $\times 10^4$	TENSION EXERTED BY MUSCLES IN GRAMS
January 17	0.75	0	74	100	267
		0	84	111	350
		0	70	93	312
February 16	1.15	0	140	243	725
		0	105	183	675
		0	90	156	700

Table III leaves no doubt as to the efficiency of the apparatus. Here we see that the muscles may be heated to 0.024° temperature by the heat of their contractions and independently of any heat of electrical origin.

In table IV one may see what, roughly, the minimum (electrical) heating of the muscles must be in order to move the swinging system of the galvanometer. This also is shown in Table II in the protocol for March 9.

If the dead cross-striated muscles after killing are heated with known amounts of electricity we again demonstrate the

[16] This value is calculated from the calibration of the instrument on the dead muscle, as already described.

sensitivity of the apparatus and obtain a value for the s.d. deflection of the galvanometer in terms of calories or (if the weight and sp. heat of the muscles be known) in terms of degrees temperature. This may be seen in the brief excerpt of the protocols as shown in table IV.

The data exhibited in tables II and IV have been purposely chosen to show one other fact. It will be noticed that whenever there is a measurable deflection the value per scale division deflection of the galvanometer seems to be somewhere between 0.0001° and 0.0003°. But the minimum increase in temperature of the muscle necessary to give a deflection is considerably more than this average value of 0.0001° or 0.0003°. Furthermore

TABLE IV

Cross-striated muscle

Passing electric current through dead muscles to calibrate value of galvanometer deflection in terms of degrees temperature, C.

DATE OF PROTOCOL, 1914	WEIGHT OF MUSCLE IN GRAMS	H. P. DUE TO ELECTRIC CURRENT IN CAL. $\times 10^6$	DITTO IN DEG. C. $\times 10^6$	DEFLECTION OF GALVANOMETER IN S. D.	VALUE OF 1 S. D. OF GALV. DEFL. IN DEG. C. $\times 10^6$
January 17	0.75	9.2	12.2	0	0
		25.8	34.4	30	0.86
February 16	1.15	18.1	15.7	5	3.60
		27.2	23.6	35	0.77

there is a tendency for the value per scale division deflection to increase as the amount of muscle heating decreases and vice versa, as is indicated somewhat in all the examples given in both tables II and IV, and very sharply shown in the protocol for March 9, table II.

The explanation of this phenomenon is simply that the moving system of the galvanometer, in spite of its small mass (about 30 mg.) has considerable inertia. The sensitivity of the galvanometer, other things being equal, is influenced chiefly by the distance of the coils from the swinging system of magnets and the position of the damping magnet. Both of these latter factors were varied during the investigation so that the minimum heating required to set the swinging system in motion is not constant in the various protocols. (At no time however was the instrument set at its greatest sensitivity). The requisite

minimum heating is most nearly approached in the protocols shown in Table IV; 0.0012° increase seems to be insufficient, while 0.0015° increase gives a measurable deflection. Again the protocol for March, Table II, shows an increase of 0.0023° insufficient to move the spot.

This requisite minimum heating was not always accurately determined. The fact of its existence is sufficiently clear to lead one to the conclusion that smooth muscle *may* produce heat while contracting, and that the amount may be too small to overcome the inertia of the swinging system.

Now however small this requisite minimum of the galvanometer may be, it is quite clear that so long as it is greater than the average value per s.d. deflection as determined by larger quantities of heat, the argument still holds good, the smooth muscle's heat production may be just too small to set the galvanometer in motion.

For this reason it was decided not to bother about the determination of this "requisite minimum" for each experiment but to resort to some method by which the galvanometer is set in motion (that is by which its requisite minimum is provided whether the muscle itself produces any heat or not).

This object it is evident would be accomplished by sending the stimulating electrical current directly through the whole of the muscle under observation instead of through its nerve or adjacent tissues (neck of bladder). The heat produced in the muscle by the stimulating current could be accurately known and could be taken sufficiently large to supply this very requisite minimum. Observations upon the dead muscle, passing the current in like manner, would give the deflections due to heat of electrical origin only, and a basis for comparing the deflections obtained on the live muscle, as is formally set forth above in the discussion of direct stimulation, see pages 15–16.

Obviously if smooth muscle produces heat only of the magnitude of a very few scale divisions one ought to be able to detect it then by means of the method of direct stimulation of muscle.

Results of direct stimulation. To demonstrate how the method operates the following observations on a muscle known to produce heat of its own are shown.

(a) A pair of surviving gastrocnemius frog muscles, of 0.72 grams weight were put on the thermopile March 9, 1914, and stimulated directly:

Heat of electrical origin (h_e) in calories $\times 10^4$	104.00
Deflection of galvanometer in (cm.) s.d., ($h_e + h_m$)	300.00

(b) The same killed in position:

Value of h_e in calories $\times 10^4$	255.00
Deflection of galvanometer in (cm.) s.d	300.00
Value of 1 scale division deflection in calories $\times 10^4$	0.85

(c) Value of the total deflection of 300 s.d. observed on the live muscle in calories $\times 10_e$ $(h_e + h_m) = 255$. As is seen in (b) to obtain a deflection of the galvanometer (when the muscle was dead) equal to that obtained on the live muscle the electrical heating had to produce 0.0255 calories.

The value of the electrical heating plus the muscular heating $(h_e + h_m)$ then in the case of the live muscles, when the deflection was 300 s.d., must have been equal to 0.0255 calories. The electrical heating (h_e) of the stimulated live muscle alone was 0.0104 calories. The muscular heating (h_m) therefore must have been the difference between 0.0255 and 0.0104, or 0.0151 cal.; i.e. since $h_e + h_m = 0.0255$ cal. and $h_e = 0.0104$ cal., $h_m = 0.0151$ cal.

From the muscle's weight (taking the sp. heat = 1) one calculates the increase in temperature due to the heat of muscular origin to have been 0.0209°C.

The tension exerted to produce this increase of temperature was 475 grams. One gram tension corresponds to an increase then of 4.4×15^5 degrees temperature.

In the experiment of May 18, 1914, a pair of frog's sartorius muscles were placed in the thermopile; weight, 0.265 grams; isometric contractions. The readings during *direct stimulation* are as follows:

(a) *Surviving muscles:*

Heat due to electric current only (h_e) in calories	0.0041
Total deflection of the galvanometer in cm. s.d.	76.0
Max. tension exerted by muscles in grams	7.5

(b) *Dead muscles,*

Heat due to electric current only (h_e) in calories	0.0716
Total deflection of the galvanometer in cm. s.d.	80.0
Value of 1 s.d. deflection of the galvanometer in calories	0.000895

From (b) we see that 1 s.d. deflection has a value of about 0.000895 calories. The total deflection of the galvanometer in (a) then has a value of $h_e + h_m = 0.000895 \times 76 = 0.068$ calories. From (a) $h_e = 0.0041$ calories, therefore $0.0680 - 0.0041 = 0.0639$ calories $= h_m$.

The heat of muscular origin thus was 0.0639 calories, or an increase in temperature of $\frac{0.639}{0.265}$, or 0.24°C. Since the tension in this case was only 7.5 grams, 1 gram of tension for the sartorius muscle represents 0.032°C.

The above examples illustrate sufficiently the differential method for the measurement of small quantities of heat in tissues. As is seen the muscle heat may be many times greater than the heat of electrical origin.

Indeed an adequate stimulating current of short duration may itself produce so little heat that the galvanometer would not be able to register it. And yet the resulting muscle heat accompanying the sudden development of tension may be so great as to cause the spot to fly off the scale. In the experiment of May 18, the first reading gives a value of 0.00027 calories for h_e and 0.0397 calories for h_m.

The results of applying this method to the turtle's bladder muscle so far have not been conclusive, nor does repetition of the experiments improve their character.

Nevertheless typical data are here submitted since they are all stamped in one way, namely, in being unlike the results obtained with cross-striated muscle.

Experiment of January 22, 1914. Turtle's bladder, direct stimulation; isometric contraction.

(a)	The surviving muscle:					
	Value of h_e in calories $\times 10^4$	37.7	139.0	185.0	111.0	134.0
	Deflections of galvanometer in cm. s.d.	0.0	80.0	120.0	64.0	36.0
	Tension exerted in grams	12.0	50.0	48.0	48.0	33.0
(b)	The dead muscle:					
	Value of h_e in calories $\times 10^4$		252.0	447.0	126.0	112.0
	Deflections of galvanometer in cm s.d.		65.0	120.0	60.0	45.0
	Value of 1 s.d. deflection in calories $\times 10^4$		3.8	3.8	2.1	2.5

(c) Values of galvanometer deflection in (a), calculated from value of 1 s.d. in (b), expressed in calories $\times 10^4$, being sum of $h_e + h_m$	0.0	304.0	444.0	134.0	90.0
Values of h_e, from (a)	37.7	139.0	185.0	111.0	134.0
Subtracting gives h_m in calories $\times 10^4$	−37.7	+165.0	+259.0	+ 23.0	− 44.0

Experiment of March 9, 1914. (a) Turtle's bladder, muscle; isometric contractions of living muscle; weight of muscle 0.82 grams.

Number of reading	1	2	3	4	5
Value of h_e in degree C. $\times 10^4$	2.0	23.0	88.0	216.0	301.0
Deflection of galvanometer in cm. s.d. (due to $h_e + h_m$)	0.0	0.0	0.0	+260.0	+360.0
Tension exerted, in grams	0.0	50.0	105.0	122.0	120.0
(b) Repeating the experiment on the dead muscle:					
Number of reading	1	2	3	4	5
Value of h_e in calories $\times 10^4$	19.0	72.0	118.0	178.0	247.0
Increase in temperature of muscle in degrees C. $\times 10^4$	23.0	88.0	140.0	216.0	301.0
Total deflection of the galvanometer due to h_e in cm. s.d	0.0	24.0	80.0	200.0	300.0
Value of 1 s.d. deflection of the galvanometer in degrees C. $\times 10^4$	0.0	3.5	1.7	1.0	1.0
(c) From the values in the last rubric of (b) we may give the galvanometer deflections in (a) the following values of $(h_e + h_m)$:					
Number of reading	1	2	3	4	5
Value of galvanometer deflection in (a) in degrees C. $(h_e + h_m) \times 10^4$	0.0	0.0	0.0	+260.0	+360.0
Values of h_e in (a), in degrees C. $\times 10^4$	+2.0	+23.0	+88.0	+216.0	+301.0
Values of h_m in degrees C. $\times 10^4$ $= (h_e + h_m) - h_e$	−2.0	−23.0	−88.0	+44.0	+59.0

Experiment of June 1, 1914. Turtle's bladder, weight, ca. 1 gram; isometric contraction; direct stimulation; value of 1 s.d. deflection of the galvanometer from readings on dead muscle, 0.00026 calories.

Value of h_e in calories $\times 10^4$	150.0	150.0	10.9	100.0
Deflection of galvanometer in cm. s.d. due to $h_e + h_m$	40.0	80.0	0.0	30.0
Value of same in calories $\times 10^4$ (1 s.d. = 0.00026 calories)	101.0	208.0	±0.0	78.0
Value h_m (muscle heat) in calories $\times 10^4$	−46.0	+58.0	−10.9	−22.0
Tensions exerted in grams	18.0	?	7.0	13.0

Experiment of July 10. Ditto.

Values of h_m (muscle heat) in calories $\times 10^4$				
	−5.3	−47.0	−41.0	−138.0

These negative values are offset by nearly all positive values for h_m in the

Experiment of July 15. Ditto.

Values of h_m in calories $\times 10$					
	+16.0	−24.0	+32.0	+95.0	+123.0

A glance at the values of h_m in the foregoing protocols is sufficient to indicate the uncertainty of their meaning. Controls were repeatedly made on the dead muscles and there the deflections and their heat values were constant. Furthermore it must be remembered that the muscles were attached to isometric levers. Movement of the tissue over the thermopile junctions was therefore practically excluded. Indeed trial tests with isotonic levers, where the muscles shorten freely, and sometimes spontaneously, showed no such irregularity of deflections. It ought to be recorded here that during spontaneous rhythmical contractions of the turtle's bladder while astride the thermopile no corresponding rhythmical heat changes could be detected.

Summary of results. To sum up the results so far recorded we may say the following.

1. When stimulating smooth muscle indirectly the results were uniformly constant in the absence of any measurable amounts of heat production accompanying development of tension.

2. When stimulating by the direct method, by the same apparatus, development of tension in smooth muscle was accompanied now with positive heat changes, now with negative heat changes. But the same apparatus when measuring heat changes in dead muscles (heated electrically), or in live cross-striated muscle stimulated directly gave quite uniform and positive results.

3. It was hoped that the methods of stimulating the muscle directly would enable one to detect more delicate amounts of heat change than by the method of indirect stimulation, the method being a sort of differential method. But this has not

yet been, demonstrated, unforeseen and inexplicable factors having been apparently introduced.

Only future experiments (now under way) carried out with greater refinements and more rigorous controls can answer the question with more satisfaction—Does smooth muscle produce heat when it contracts? Meanwhile it may not be unprofitable to take an inventory of the situation and give the results so far obtained a fuller discussion.

DISCUSSION OF RESULTS

A possible explanation of the negative results in this investigation may be found in the consideration that the heat produced in smooth muscle may be just as great as it is in cross-striated but that it is produced much more slowly, corresponding to the known slowness of its tetanus development and its prolonged latent period. The time required for the contraction is thus great enough to allow the heat developed to be dissipated by conduction, radiation, etc., before an increase of temperature can be measurably effected.

But even if the heat produced be dissipated and thereby escape detection, it is difficult to see how the same causes could operate in rendering the accompanying gaseous exchanges, as the investigation of Bethe and Parnas have shown, vanishingly small. The heat may be lost, but how could the consumption of O_2 be made good, or the increase output of CO_2 escape chemical detection.

In his discussion of the thermodynamics of muscle O. Frank (1904) maintains that the difference in energy expenditure of muscles is due to the difference in the tetanizing *rates* normally required of their appropriate nerve centres to put them into a contracted state. The requisite tetanizing rate to put smooth muscle into an increased tetanus condition being the lowest of any muscle, the expenditure of energy would be accordingly also the lowest. Since this statement was made the facts upon which the argument was based have been greatly modified by more recent experiments.

Campbell (1888) had already shown[17] that the tetanizing rate

[17] Campbell, J. G.: Studies from the Biological Laboratory of the Johns Hopkins University, 1888, iv, p. 123.

in the terrapin, Malacochemmys palustris, was tremendously influenced by temperature. At 4° the rate was found to be as low as 1 stimulus per second, and at 28° as high as 34 per second. Applying the usual temperature coefficient expected in such processes, 2, would render this rate 64 at 38°C.

It was left for Piper[18] to show that in general the tetanizing rate of cross-striated muscle under normal innervation is a function of the temperature rather than of position in the phylogenetic scale. At 37°C. turtle's retractores nuchae have a normal rhythm as great as the flexores digitorum of man.

It may be added here that a little observation in mid-summer will convince one that the buzzing of an insect's wing reaches its highest pitch (probably the classical number 300 vibrations) only on the warmest days. In any event the rapid contraction of the wing muscles of an insect in flight is not properly a tetanus at all.

However Fucks (1908)[19] succeeded in photographing the active current of a smooth muscle as it went into contraction in response to reflex stimulation, and, showed the action current to be really a single mono- or diphasic curve according to the leads. Smooth muscle therefore unlike most cross-striated muscle contractions when normally innervated may exert a continued increase of tension, a tetanus, in response to a single nervous impulse.

So the graded scale of diminishing tetanizing rates used in Franck's argument dwindles down to two rates, a fairly rapid one for cross-striated muscle, say 60 per second at 37°C. and a very slow one for smooth muscle, say 1 per second at 37°C.

But in spite of this transformation in the facts at the basis of Franck's argument the main point of his contention thus far still holds water. But only thus far, for there are other new data that must be reckoned with.

The decerebrate rigidity described by Sherrington[20] is now

[18] Piper, H.: Archiv für Anatomie und Physiologie, physiologische Abteilung, 1910, p. 207; Archiv für die gesammte Physiologie, 1907, cxix, p. 301.

[19] Fucks, R. F.: Ber. d. Deutschen physiol. Gesellsch. Würzburg, 1909. See also Zentralblatt für Physiologie, 1909, xxiii, p. 296.

[20] Sherrington, C. S.: The Integration of the Nervous System. New York, 1906, p. 89, etc.

generally well known. It is a real tetanus condition of the cross-striated muscles, a tetanus due to nerve impulses sent out over the usual nerve paths. Buytendyk,[21] working in Professor Cremer's laboratory, was able to measure the tetanizing rate of these muscles in the decerebrate preparation. The rate was found to be of the order found by Piper and his pupils for other skeletal muscles under normal conditions. Indeed it was somewhat higher, 70 to 90 per second.

According to O. Franck then the cat's muscles when in the condition of decerebrate rigidity ought to be undergoing a greatly increased rate of metabolism.

The experiments of Roaf[22] however, do not bear out this prediction. Measurements of the gaseous exchanges of cats in decerebrate rigidity show that the output of CO_2 is not appreciably more or less than when the rigidity is not present, and that the O_2 consumption "is only slightly greater."

One more fact militating against the hypothesis of a correspondence between tetanizing rate and energy expenditure, and we have done. The underlying assumption to the hypothesis is that normally skeletal muscles do not contract unless as it were, a cataract of nervous impulses is poured in upon them. It has been shown by Hoffmann[23] and independently by Snyder[24] that at least in the knee-jerk a skeletal muscle is made to contract by a normal reflex consisting of one, and one nervous impulse only.

A careful survey of the facts (not by any means completed in this paper) forces one to abandon the vaguer views physiologists have held concerning smooth muscle. Indeed the entire energetics of cross-striated as well as smooth muscle is rapidly being thrown into another mould by the fresh and vigorous attacks such as one finds in the paper of A. V. Hill already cited and the work of Noyon and von Uexküll.[25]

[21] Buytendyk: Zeitschrift für Biologie. 1912, lix, p. 36.

[22] Roaf, H. E.: Quart. Jour. of Exp. Physiology, 1912, v, p. 31; 1913, vi, p. 393.

[23] Hoffmann, P.: Arch. für Anat. u. Physiologie, physiologische Abt., 1910, p. 223.

[24] Snyder, C. D.: This Journal, 1910, xxvi, p. 474.

[25] Noyons u. von Uexküll: Zeitschrift für Biologie, 1911, lvi, p. 139.

THE EFFECT OF THE INTRAMUSCULAR INJECTION OF PITUITARY EXTRACT ON THE SECRETION OF MILK IN THE HUMAN SUBJECT

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It has been shown by Ott and Scott (1), Schäfer and Mackenzie (2), Mackenzie (3), Herring (4), Hammond (5), Gavin (6), Hill and Simpson (7), and others, that the administration of watery or saline extract of the posterior lobe of the pituitary body, by intravenous, intramuscular or subcutaneous injection, leads to an increased secretion of milk by the mammary gland.

Ott and Scott (1910), using the goat as their experimental animal, found that within one minute of the beginning of the injection of the extract into the ear vein, the flow of milk started.

A few months later Schäfer and Mackenzie confirmed the observations of Ott and Scott. On lactating cats and dogs it was found that the flow began after a latent period of twenty seconds and lasted from three to four minutes following each injection. They observed also that the effect of a second dose was much less than that of the first, and if a sufficient interval was not allowed, it failed altogether to produce any action. The anterior lobe extract was inactive, as also alcoholic extracts of the posterior lobe.

Mackenzie (1911), continuing the work, found that the galactagogue substance is present in the pituitary body not only of lactating females but also of non-lactating females and of males. It is not even specific to mammals being present also in birds, and Herring (1913) has demonstrated that extract of elasmobranch (skate) pituitary leads to a secretion of milk in the lactating cat.

Gavin (1912), in the cow, tried to determine whether the total amount of milk produced in the twenty-four hours, or its quality, is influenced by the administration of pituitary extract. The results were negative; no increase in the total quantity of milk was found, and there was no change in its composition.

Schäfer, in 1912, published the results of some observations made on the human subject. A young married lady, in the fifth month of lactation, was given four pituitary injections into the triceps humeri, at intervals of two or three days. In the words of the patient herself the general result was as follows:

> The first injection produced no effect; on each of the three subsequent occasions there was an immediate effect; I could feel the milk coming in with a tingling sensation. But so far as I can judge, no lasting effect. I judge this from the fact that though there was more milk than usual for the baby for the meal following the injection, I had to wait a long time before I could feed him again, and in spite of the injections, my milk is gradually getting less in amount. It had, however, very decidedly begun to diminish before the injections were started.

The quality of the milk was not tested.

With regard to the effect of pituitary extract on the composition of the mammary secretion, Mackenzie (3) noticed an increase in the fat content of cat's milk following injection, but no change in lactose.

Hammond (1913) observed a similar change in the goat.

Hill and Simpson (1913), also in the goat, observed a marked increase in the amount of milk yielded by the mammary gland fifteen minutes after injection. This increase, however, was followed by a corresponding decrease below the normal at the next milking period several hours later. There was a marked rise in the fat content of the milk secreted after injection. According to Hammond there was a sudden, compensatory falling off in the percentage of fat at the next milking, but Hill and Simpson did not find this to be the case. The solids-not-fat were not affected.

Hill and Simpson (8) (1914) obtained similar results in the cow.

PRESENT INVESTIGATION

The present communication deals with the effect of pituitary extract on the quantity and composition of milk in man.

The subject of these experiments was a young married woman, twenty-four years of age, in perfect health, who was nursing her second child (a girl) born on December 31, 1913.

On May 19 (1914), at 8.10 p.m., the baby was nursed from both breasts, presumably emptying them. At 9.10 p.m., one hour later, 1 cc. of Parke, Davis & Company's pituitrin (equivalent to 20 mgm. of the dried infundibular portion or to 0.4 gram of fresh gland) was injected into the biceps humeri of the left arm. Almost immediately afterwards—in about twenty seconds—the patient could feel the milk coming into the breasts, as she described it. The sensation was something between a tickle and a sting and was similar to that experienced when the baby begins to nurse. At 9.20 p.m. the milk began to be removed from both mammae with a breast (suction) pump, the process occupying about five minutes, when 50 cc. of milk were obtained, testing 6 per cent of fat.

On three days immediately preceding this experiment—May 15, 16, and 18—under exactly the same conditions, except that no injection was made (the baby was nursed at 8.10 p.m. and the glands pumped dry at 9.20 p.m.), the following amounts were yielded, giving fat contents as stated in the table.

	Amount cc.	*Fat per cent*
May 15	7	3.7
May 16	20	2.8
May 18	13	3.8

On the day following the injection there appeared to be some diminution in the amount of milk the child was able to obtain. On that day also the mother noticed that the baby had several loose motions, whereas, prior to this, she had a tendency to constipation requiring opening medicine periodically. This would seem to indicate some change in the composition of the milk.

On May 20, at 8.10 p.m., the baby was nursed and the breasts pumped dry at 9.20 p.m., as before, except that no extract was administered, when the yield was only 10 cc. with 5.7 per cent of fat.

On May 21, with the same procedure as in the first experiment, 1 cc. was injected as formerly, and 70 cc. of milk were obtained containing 4 per cent of fat. On this occasion the patient stated that she experienced distinct colic pains shortly after the injection. She recollected that she had felt similar pains during the first experiment but had attributed them to some other cause and had not thought it worth while mentioning the fact at the time.

July 2, the baby was nursed at 8 p.m. and 1 cc. pituitrin injected into the biceps at 9.14 p.m. Both breasts were pumped dry at 9.24 p.m. and yielded 75 cc. of milk giving 4.9 per cent of fat. On this occasion the milk was felt "coming in" thirty seconds after the injection and the abdominal pain was experienced about two minutes later.

July 3. Baby nursed at 8 p.m.; no injection; 9.30 p.m. both breasts pumped dry; yield 18 cc. testing 3.6 per cent of fat. As on former occasions the baby had a slight attack of diarrhoea.

July 4. Baby nursed at 8 p.m. and at 9.30 p.m. 20 cc. of milk were obtained testing 2.2 per cent of fat.

July 5. After a similar procedure the glands yielded only 10 cc. at 9.25 p.m., containing 3.5 per cent of fat.

The results of the three experiments, with the controls, are given briefly in the subjoined table.

1914	GLANDS DRIED BY BABY	MILK WITHDRAWN BY BREAST PUMP	AMOUNT	FAT
	p.m.	*p.m.*	*cc.*	*per cent*
May 15	8.10	9.20	7	3.7
16	8.10	9.20	20	2.8
18	8.10	9.20	13	3.8
*19**	*8.10*	*9.20*	*50*	*6.0*
20	8.10	9.20	10	5.7
*21**	*8.10*	*9.20*	*70*	*4.0*
July *2**	*8.00*	*9.24*	*75*	*4.9*
3	8.00	9.30	18	3.6
4	8.00	9.30	20	2.2
5	8.00	9.25	10	3.5

* On these days 1 cc. of Parke, Davis & Co's pituitrin was injected into the biceps humeri ten minutes before the milk began to be withdrawn from the breasts by suction.

SUMMARY

1. In a young, healthy, married woman, nursing her second baby, in the fifth month of lactation, injection of 1 cc. of Parke, Davis and Co.'s pituitrin, equivalent to 20 mgm. of the dried infundibular portion of the gland, was followed, in ten minutes, by a marked increase in the amount of milk secreted.

2. After a latent period of from twenty to thirty seconds the mother could feel the milk "coming into the breasts," and a few minutes later abdominal pains were experienced, presumably due to increased intestinal peristalsis.

3. The milk withdrawn after injection was rich in fat, the average figure for the three experiments being 5.5 per cent as compared with 3.4 per cent for three days when no pituitrin was administered. In the milk yielded twenty-four hours after injection the fat content was still somewhat above the normal.

4. Although no quantitative estimations were made, judging from the mother's experience in nursing the child, it would appear that the quantity of milk obtainable by it on the morning after the injection was diminished.

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